Heliyon 10 (2024) e34846

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effects of actinomycetes on the growth, antioxidant and genes expression in *Fritillaria taipaiensis* P. Y. li

Xiaotian Kong [a,b], Liang Han [c], Liqin Yang [d], Zhifen Shi [b,e], Jiaqi Lang [b], Mingyan Ye [b], Bo Xiao [c,**], Xubing Chen [a,***], Nong Zhou [a,b,e,*]

[a] College of Pharmacy, Dali University, Dali, 671000, China
[b] College of Food and Biological Engineering, Chongqing Three Gorges University, Chongqing, 404020, China
[c] Chongqing Institute of Medicinal Plant Cultivation, Chongqing, 408435, China
[d] Shangri La City Agriculture and Rural Bureau, Shangri La, 674499, China
[e] College of Modern Chinese Medicine Industry, Chengdu University of Traditional Chinese Medicine, Chengdu, 611130, China

ARTICLE INFO

Keywords:
Antioxidant competence
Fritillaria taipaiensis
Gene-related expression
Growth
Growth-enhancing actinomycetes
Microbial fertilizer

ABSTRACT

F. taipaiensis P. Y. Li represents a significant asset within traditional Chinese medicinal flora, though it confronts the challenge of germplasm deterioration during its cultivation phase. This study aimed to discern the implications of single strains or combinations of diverse growth-promoting actinomycetes on the growth metrics, antioxidant competence and pertinent gene expression in the leaves of *F. taipaiensis*. The result revealed that the malondialdehyde content within the plant's leaves notably diminished in the treatment groups compared to the CK group, with the S6 group showcasing the most pronounced malondialdehyde reduction, amounting to approximately one-third of the CK's value. Leaf area, length and width peaked in the S5 cohort, registering values 4.55, 2.46 and 1.85 times surpassing the CK group. Concurrently, plant height and stem thickness were maximal in the S6 group, being 2.29 and 1.75 times that of the CK group, whereas leaf thickness reached its zenith in the S7 group, marking a 2.17-fold elevation compared to the CK. Photosynthetic pigments, soluble sugars and soluble proteins in the leaves, exhibited augmentation across the inoculated groups to varying magnitudes. Specifically, the S5 group was superior in photosynthetic metrics and pigments, while the S6 group manifested the highest soluble sugar concentration, which was 1.35 times that of the CK. The S3 group demonstrated the pinnacle of soluble protein content, an impressive 5.86-fold increment relative to the CK group. The enzymatic activities of superoxide dismutase, peroxidase and catalase, along with their affiliated gene expressions, were observably augmented in the inoculated groups, with the S5 group standing out. To encapsulate, the actinomycete inoculation holds potential in fostering the growth and maturation of *F. taipaiensis*, amplifying its environmental resilience. The revelations from this study extend valuable insights for the judicious choice of microbial fertilizers in the cultivated propagation of *Fritillaria taipaiensis* P. Y. Li.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: 362328113@qq.com (B. Xiao), chenxubing@dali.edu.cn (X. Chen), erhaizn@126.com (N. Zhou).

https://doi.org/10.1016/j.heliyon.2024.e34846
Received 3 December 2023; Received in revised form 29 June 2024; Accepted 17 July 2024
Available online 17 July 2024

1. Introduction

Fritillaria taipaiensis P. Y. Li represents a perennial herbaceous species belonging to the genus *Fritillaria* within the esteemed Liliaceae family. Notably, it has a historical significance, with its dried bulbs being utilized for medicinal purposes. This plant is prominently featured as one of the foundational source plants for *F. cirrhosa* (Chuan beimu) as documented in the Pharmacopoeia of the People's Republic of China (2020 Commission) [1]. The *F. taipaiensis* is known for its bitter taste and slight chilliness, and it is believed to have functions such as clearing phlegm, moistening the lungs, relieving coughs, reducing asthma, dispersing clots, and resolving abscesses. It has been widely used in clinical applications for conditions such as lung-heat induced dry cough and lung yin deficiency in the provinces of Shaanxi, Gansu, Ningxia, Sichuan, and Chongqing in China [2]. As the only type of *F. cirrhosa* (Chuan beimu) suitable for cultivation at relatively low altitudes, the market prospects for *F. taipaiensis* are promising. In recent years, due to over-harvesting and environmental degradation, wild resources of *F. taipaiensis* have become extremely scarce, making artificial cultivation an effective means to address the market supply-demand contradiction [3]. Although the technology for artificial cultivation is gradually maturing and has to some extent alleviated scarcity, proper fertilization and soil quality improvement in cultivation areas are crucial for improving the yield and quality of the medicinal materials.

The paradigm of the "inter-root" concept can be traced back to 1904, crediting Lorenz Hilnter [4], an eminent German microbiologist. He meticulously characterized microorganisms proximal to the soil as inter-root microorganisms. Plant grows promoting rhizobacterias (PGPRs) are a group of beneficial microorganisms that colonise plant roots and positively affect plant growth by increasing nutrient accumulation in the inter-root zone, promoting plant growth, inhibiting damage by pathogenic microorganisms and increasing plant resistance [5]. Actinomycetes hold pivotal roles within the soil microbial milieu, constituting an impressive 10–50 percent of the overall microbial consortium. Among the diverse microorganisms populating the rhizosphere, actinomycetes are lauded for their unparalleled proficiency in facilitating plant growth, a testament to their myriad beneficial attributes [6]. These microorganisms, by secreting compounds such as indoleacetic acid (IAA), generating iron chelators, solubilizing phosphorus and fixing nitrogen, furnish essential nutrients, setting the stage for optimal plant growth. Concurrently, they mount a defense against pathogenic microorganisms through the synthesis of antimicrobial compounds and enzymes like chitinase, cellulase and protease, thereby augmenting plant resistance [7]. Given these growth-enhancing traits, harnessing actinomycetes as biopesticides emerges as a cost-effective and environmentally benign approach. It is necessary to explore microbial resources for promoting plant growth, biological control and reducing the use of high pollution chemical fertilizers and pesticides in order to realize the sustainable development of traditional Chinese medicine [8].

In recent years, the related studies on *F. taipaiensis* are mainly focused on the investigation of germplasm resources [9–11], medicinal value [12,13], medicinal active components [14–16] and production technology, while there are relatively few reports on *F. taipaiensis* and microorganisms. Mu et al. [17]found that the contents of alkaloids and other effective components in *F. taipaiensis* were consistent with the number of culturable bacteria and actinomycetes in the rhizosphere. Zhang et al. [18] investigated that inoculation with arbuscular mycorrhizal fungi such as *Glomus* can improve the yield and quality of *F. taipaiensis*. Shi et al. [19] studied that inoculation with phosphate solubilizing bacteria can significantly increase the expression of genes related to physiology, biochemistry and protective enzyme system, and promote the growth of *F. taipaiensis*. However, scholarly literature remains bereft of insights into the ramifications of single strains or combinations of diverse growth-promoting actinomycetes on the growth trajectories and developmental dynamics of *F. taipaiensis*.

In this experiment, bulbs of *F. taipaiensis* were selected as experimental materials, single rhizosphere growth-promoting actinomycetes and mixed rhizosphere growth-promoting actinomycetes were applied to study the effects of mixed actinomycetes and single actinomycetes on the growth and development of *F. taipaiensis*. Key observational parameters included the growth parameters, photosynthetic traits, antioxidant enzyme activities, and relative gene expressions of *F. taipaiensis*. The purpose of this study is to find out the most effective single strain or combination of growth-promoting actinomycetes needed for artificial cultivation of *F. taipaiensis*, and to provide reference basis for the application of growth-promoting actinomycetes in the production of *F. taipaiensis*.

2. Materials and methods

2.1. Materials

Fresh bulbs of *F. Taipaiensis* were collected from mature 4-year-old plants at the Hongchi Dam Scenic Area cultivation base in Wuxi County, Chongqing Municipality. The specific geographical coordinates of the collection site were 108°56′31.92″E longitude and 31°38′7.80″N latitude, at an elevation of approximately 2000.7 m. This locale is characterized by a quintessential tri-dimensional alpine mountain climate, with summer temperatures averaging 17 °C or lower, and an expansive forest and grass cover encompassing over 85 percent of the area. To ensure the stability and homogeneity of the germplasm resources, the bulb source was conserved as individual plants and subjected to routine processing and management. The acquired sample was authenticated as a fresh bulb of *F. taipaiensis* P. Y. Li, a constituent of the Liliaceae family by Professor Zhou Nong of Chongqing Three Gorges College. During the cultivation period, the routine management was carried out according to the indoor cultivation of *F. Taipaiensis* plants. The experimental strains incorporated three actinomycete species: *Streptomyces lavendulae* (*S. lavendulae*), *Streptomyces fradiae* (*S. fradiae*) and *Streptomyces zaomyceticus* (*S. zaomyceticus*). These were isolated, cultured, and activated by our research team, demonstrating significant growth-promoting efficacy in the inter-root soil of *F. Taipaiensis*. These cultures were incubated on a shaker set at a stable 28 °C for 5 days (180 rpm) using Gauze's Synthetic Broth Medium. The actinomycete suspension was subsequently diluted to a concentration of 2×10^8 CFU/mL utilizing sterile water, yielding the inoculum.

2.2. Experimental design

Pot experiments were orchestrated at the Hongchiba cultivation base in Wuxi, Chongqing, China, commencing in early October 2022. The potting mix comprised yellow loam, sand and organic fertilizer in a 2:1:1 proportion. This mixture was sieved through a 2 mm mesh, intermittently sterilised at 121 °C for 2 h in an autoclave sterilisation pot and subsequently cooled and stored hermetically. The potting containers were plastic pots 18 cm in diameter and 20 cm in height, pre-cleaned thrice using anhydrous ethanol. To perform the experiment, eight distinct groups were established, encompassing 7 treatment groups (S1–S7) and a non-inoculated control group, CK. In the treatment groups, the three dominant species of actinomycetes were inoculated either singly, in pairs, or as a three-species. Specifically, the S1 group was inoculated with *S. lavendulae*, the S2 group with *S. fradiae,* the S3 group with *S. zaomyceticus*, the S4 group was inoculated with *S. lavendulae* and *S. fradiae*, the S5 group was inoculated with *S. lavendulae* and *S. zaomyceticus*, the S6 group was inoculated with *S. fradiae* and *S. zaomyceticus*, while the S7 group was inoculated with *S. lavendulae*, *S. fradiae* and *S. zaomyceticus*. In March 2023, the inoculant was applied in a uniform layer to the fibrous roots of *F. Taipaiensis* under the topsoil. Each treatment replicated across 10 pots, each embedded with 5 well-growing *F. Taipaiensis* bulbs, with an inoculum dosage of 90 mL per pot. All treatments were inoculated twice during the cultivation period. Post-inoculation, the plants were nurtured under ambient conditions (with natural illumination) and received standard care throughout their growth trajectory.

2.3. Growth indicator measurements

In April 2023, when the leaves of *F. taipaiensis* were flourishing, a sunny day (11:00~13:00) was selected, and when the stomatal opening of the leaves reached its maximum value, from each treatment group, the leave of *F. taipaiensis* with good growth and no pests and diseases were randomly selected from each pot. Under the condition of not picking leaves, the length and width of the middle of the leaves of the same part of the leaves of the different plants were measured by a ruler or vernier calipers to calculate the leaf area. Vernier calipers were used to measured plant height, stem thickness and leaf thickness. Each indicator was repeated three times. Then, following the method of Zhao Xin et al. [23], a photosynthesiometer was used to determine the gas exchange parameters of the corresponding *F. taipaiensis* leaves. Finally, the leaves of the same *F. taipaiensis* plant were extracted to determine the physiological and biochemical indicators.

2.4. Biological activity assay

The content of photosynthetic pigments within the leaves of *F. taipaiensis* was executed following the protocol established by Shu Zhan et al. [20], with stringent light-avoidance throughout the process. The thiobarbituric acid colourimetric method [21]was used to determine the content of malondialdehyde (MDA) and soluble sugars in the leaves. The colorimetric procedure using the Coomassie brilliant blue G250 [21]was employed for soluble protein content in the leaves. Superoxide dismutase (SOD) activity was gauged using the azidotetrazolium photochemical reduction method [22], where a 50 percent inhibition of NBT photochemical reduction was equated to one unit of enzymatic activity (U). Peroxidase (POD) activity was determined by the guaiacol method [23], and an increase in absorbance (A) of 0.01 per minute was defined as one unit of enzyme activity U. Catalase (CAT) activity in leaves was determined by UV spectrophotometry [23]and a decrease of 0.1 per minute in A_{240} nm was defined as one enzyme activity unit U.

2.5. Antioxidant enzyme gene expression analysis

Total RNA was extracted from Taebaek mussel leaves using TRIzol® Plus RNA Purification Kit (Invitrogen). Complementary DNA (cDNA) was reverse transcribed using SuperScript™ III First-Strand Synthesis SuperMix Kit (Invitrogen). The relative expression of *SOD*, *POD* and *CAT* gene were determined by Real-time PCR using the Power SYBR® Green PCR Master Mix Kit (Roche) and the LQuantstudio multiplex real-time fluorescence quantitative PCR instrument (life technologies, USA). The reaction system was 20 μL and here were the reaction conditions: 95°Cfor 1 min, 95 °C for 15s, 63 °C for 25s and 40 cycles. Relative gene expression was calculated by $2^{-\Delta\Delta Ct}$ analysis using rpl16 as the internal reference gene. Each sample was repeated three times, and Primer Premier 6.0 and Beacon designer 7.8 software were used for quantitative PCR primer design, followed by synthesis. Data pertaining to Real-time PCR primers are articulated in Table 1.

Table 1
Real-time PCR primers information.

Gene	Primers Sequences (5′-3′)
SOD	F:TTCAGTTTCTTAGTGACAATAGGCG
	R:GGTCTTAGTCTGGATACGGCAA
POD	F:TTTCCTTTCCATTCACCCG
	R:AAGACCCTTCCCTTTGTTCG
CAT	F:TATTCCACAACAACGAAAGCAC
	R:GGACCCGAATCCGTTAGTATG
rpl16	F:TTCGTGCTACATTCGTAGGGTC
	R:GTTCCATTGCGGAGTTCGG

2.6. Data analysis

The experimental data were processed by Microsoft Excel 2019, the statistical analysis was conducted with SPSS27.0 software and the images were plotted with Origin 2021 software.

3. Results

3.1. Effect of inoculation with different growth-promoting actinomycetes on the growth indicators of F. taipaiensis

Inoculation with growth-promoting actinomycetes exerted an influence on the growth of *F. taipaiensis*, with several distinctions reaching statistical significance ($P < 0.05$) as depicted in Table 2. Except for S7 group, the leaf width of *F. taipaiensis*in in all treatment groups was significantly surpassed that of the CK group. Group S5 had the largest leaf width, which was 1.85 times higher than that of CK group. Excluding the S1 group, leaf stem thickness indicators of other treatment groups were higher than CK group to varying extents. The pinnacle of stem thickness was observed in the S6 group, marking a 1.75-fold increment over the CK group. Moreover, indicators like plant height, leaf thickness, leaf length and leaf area exhibited superior values across various treatment groups ensembles relative to the CK group. Compared with the CK group, S5 group had the largest leaf length and leaf area in the treatment group, which was 2.46 and 4.55 times that of the CK group, respectively. The greatest plant height among the treatment groups was in the S6 group, at 2.29 times the CK group, while Group S7 had the largest leaf thickness, which was 1.85 times higher than that in CK group. On the whole, the growth indicators of *F. taipaiensis* could be improved to different degrees by inoculating different growth-promoting actinomycetes. Furthermore, growth indicators for the S4–S7 groups treated with duo or trio actinomycete species eclipsed those of the S1–S3 groups treated with a singular actinomycete species, and the effect of S5 group was more satisfactory.

3.2. Effect of inoculation with different growth-promoting actinomycetes on photosynthetic parameters in the leaves of F. taipaiensis

The results showed that in comparison to the CK group, Net photosynthetic rate (Pn),Stomatal conductance(Gs), Transpiration rate (Tr) and Intercellular CO2 concentration(Ci) in the leaves of *F. taipaiensis* inoculated with different growth-promoting actinomycetes in the S1∼S7 groups were significantly increased, and most of the differences were statistically significant ($P < 0.05$)) as tabulated in Table 3. Among them, Pn and Tr Ci were the highest in S5 group, which were 1.48, 2.41 and 2.28 times those of the CK group, respectively. Gs peaked in the S6 group, demonstrating a 2.24-fold enhancement over the CK group. Except that the water use efficiency (WUE) of the leaves in group S4 (*S. lavendulae* and *S. fradiae*) was significantly higher than that in the CK group ($P < 0.05$). The WUE of remaining groups either paralleled or trailed the CK group, potentially attributed to the ample soil moisture conditions maintained throughout the experimentation phase.

3.3. Effect of inoculation with growth-promoting actinomycetes on the photosynthetic pigment content in tne leaves of F. taipaiensis

The experimental data revealed that the inoculation of growth-promoting actinomycetes had an effect on the content of photosynthetic pigments in the leaves of *F. taipaiensis*, with some differences being statistically significant ($P < 0.05$) in comparison with CK group. According to the results shown in Table 4, the content of chlorophyll *a* in the leaves of *F. taipaiensis* in all treatment groups was higher than that in CK group. Among them, the S5 group had the highest content of chlorophyll *a*, which was 1.22 times higher than that of CK group. With the exception of the S6 group, both the content of chlorophyll *b* and the total amount of chlorophylls in the leaves of *F. taipaiensis* exceeded those in the CK group, with the highest content in the S5 group. The content of carotenoids was elevated in all treatment groups relative to the CK group. The highest carotenoid content was observed in the S7 group, which was 1.33 times higher than in the CK group. In essence, inoculating growth-promoting actinomycetes affected photosynthetic pigments in *F. taipaiensis* leaves to varying degrees. A predominant upward trend in the total content of the three photosynthetic pigments was discernible in most treatment ensembles compared to the CK group, with the most pronounced enhancement observed in the S5 group. Overall, the application of actinomycetes resulted in an increase in the content of photosynthetic pigments in the leaves of *F. taipaiensis*.

Table 2
Effect of inoculation with different growth-promoting actinomycetes on the growth indicators of *F. taipaiensis*.

Treatments	Plant height/cm	Stem thickness/mm	Leaf thickness/mm	Leaf length/cm	Leaf width/cm	Leaf area/cm^2
CK	4.363 ± 0.375g	0.893 ± 0.111c	0.320 ± 0.026d	3.343 ± 0.059h	0.843 ± 0.040d	2.117 ± 0.135g
S1	5.643 ± 0.560f	0.873 ± 0.443c	0.450 ± 0.050c	5.880 ± 0.089g	1.310 ± 0.090b	5.783 ± 0.481d
S2	7.243 ± 0.457cd	1.183 ± 0.244abc	0.500 ± 0.076bc	6.260 ± 0.050f	1.053 ± 0.058c	4.947 ± 0.238e
S3	6.660 ± 0.303de	1.083 ± 0.051bc	0.477 ± 0.032c	6.377 ± 0.065e	1.257 ± 0.050b	6.013 ± 0.301d
S4	7.800 ± 0.272bc	1.207 ± 0.081abc	0.500 ± 0.079bc	7.517 ± 0.031c	1.520 ± 0.026a	8.567 ± 0.142b
S5	8.090 ± 0.164b	1.547 ± 0.065a	0.570 ± 0.026b	8.237 ± 0.031a	1.560 ± 0.078a	9.637 ± 0.508a
S6	10.013 ± 0.268a	1.560 ± 0.252a	0.567 ± 0.049b	8.117 ± 0.095b	1.140 ± 0.026c	6.940 ± 0.236c
S7	6.447 ± 0.190e	1.433 ± 0.099ab	0.693 ± 0.038a	6.830 ± 0.082d	0.657 ± 0.050e	3.363 ± 0.285f

Note: Data are mean ± SD. N = 3. Different letters in the same column indicate significant differences at $P < 0.05$ levels by LSD test, the same as below.

Table 3
Effect of inoculation with different growth-promoting actinomycetes on photosynthetic parameters in the leaves of *F. taipaiensis*.

Treatments	Net photosynthetic rate (*Pn*)/μmol·m^{-2}·s^{-1}	Stomatal conductance (*Gs*)/μmol·m^{-2}·s^{-1}	Transpiration rate (Tr)/ mmol·m^{-2}·s^{-1}	Intercellular CO_2 concentration (Ci)/ μmol·m^{-2}·s^{-1}	Water use efficiency (WUE)/μmol/mmol
CK	10.000 ± 0.100e	0.076 ± 0.004e	0.980 ± 0.010g	123.700 ± 3.032g	10.217 ± 0.021b
S1	10.633 ± 0.757d	0.108 ± 0.010d	1.890 ± 0.010d	144.433 ± 5.948f	5.633 ± 0.410f
S2	13.067 ± 0.115c	0.117 ± 0.009cd	2.037 ± 0.055c	172.900 ± 1.652de	6.423 ± 0.114e
S3	14.733 ± 0.208a	0.118 ± 0.003cd	2.067 ± 0.025bc	260.033 ± 2.639b	7.120 ± 0.010d
S4	13.200 ± 0.200c	0.114 ± 0.013d	1.120 ± 0.060f	171.967 ± 3.190e	11.793 ± 0.558a
S5	14.833 ± 0.208a	0.127 ± 0.005c	2.357 ± 0.040a	282.067 ± 3.213a	6.300 ± 0.104e
S6	14.000 ± 0.100b	0.170 ± 0.002a	2.117 ± 0.050b	246.500 ± 2.821c	6.613 ± 0.189e
S7	14.067 ± 0.252b	0.143 ± 0.003b	1.703 ± 0.074e	179.533 ± 5.972d	8.263 ± 0.214c

Table 4
Effect of inoculation with growth-promoting actinomycetes on the photosynthetic pigment content in tne leaves of *F. taipaiensis*.

Treatments	Chlorophyll *a*/mg·g^{-1}	Chlorophyll *b*/mg·g^{-1}	Total chlorophyll /mg·g^{-1}	Carotenoids /mg·g^{-1}
CK	0.901 ± 0.008d	0.283 ± 0.018c	1.184 ± 0.020e	0.222 ± 0.014d
S1	0.932 ± 0.033cd	0.304 ± 0.040c	1.235 ± 0.007de	0.253 ± 0.025c
S2	1.067 ± 0.011b	0.294 ± 0.053c	1.361 ± 0.042c	0.293 ± 0.026a
S3	0.953 ± 0.013c	0.324 ± 0.005bc	1.276 ± 0.010d	0.237 ± 0.003cd
S4	1.037 ± 0.004b	0.330 ± 0.004bc	1.367 ± 0.005bc	0.296 ± 0.001a
S5	1.100 ± 0.017a	0.402 ± 0.064a	1.503 ± 0.080a	0.283 ± 0.025ab
S6	0.914 ± 0.020d	0.268 ± 0.021c	1.182 ± 0.003e	0.262 ± 0.003bc
S7	1.039 ± 0.024b	0.383 ± 0.008ab	1.422 ± 0.017b	0.297 ± 0.003a

3.4. Effect of inoculation with different growth-promoting actinomycetes on the content of malondialdehyde, soluble sugars and soluble proteins in the leaves of F. taipaiensis

As shown in Table 5, the content of malondialdehyde (MDA) within *F. taipaiensis* leaves for each treatment group was diminished relative to the CK group without inoculation, and the difference with the CK group was statistically significant ($P < 0.05$). Such outcomes substantiate that the inoculation of growth-prompting actinomycetes efficaciously curtails MDA content in *F. taipaiensis* leaves, thereby attenuating lipid membrane peroxidative damage in plant cells. Remarkably, the S6 group exhibited the lowest of MDA content in *F. taipaiensis* leaves, amounting to merely about a third of the CK group. The results of soluble sugar content showed that except for the S7 group, the soluble sugar content of the leaves of *F. taipaiensis* was significantly higher than that of CK group ($P < 0.05$), suggesting that the amalgamated inoculation of three actinomycetes may repressed soluble sugar synthesis in *F. taipaiensis* leaves. The S6 group had the highest soluble sugar content, which was 1.35 times higher than that of CK group. Results of soluble protein content showed that soluble protein content in *F. taipaiensis* leaves of each treatment group was higher than that of CK group to different extents, with a significant difference ($P < 0.05$). Then, the highest soluble protein content was found in the S3 treatment group, which was 5.86 times higher than that in the CK group. The differences among the three groups S4, S5 and S6 were not statistically significant, indicating that the effects of the three treatments were generally comparable.

Table 5
Effect of inoculation with different growth-promoting actinomycetes on the content of malondialdehyde, soluble sugars and soluble proteins in the leaves of *F. taipaiensis*.

Treatments	MDA/μmol·g^{-1}	soluble sugar/μmol·g^{-1}	soluble protein/mg·g^{-1}
CK	0.034 ± 0.004a	0.602 ± 0.014e	4.968 ± 0.693f
S1	0.027 ± 0.003b	0.772 ± 0.003b	19.495 ± 0.356d
S2	0.024 ± 0.003bc	0.682 ± 0.019d	15.271 ± 0.135e
S3	0.022 ± 0.002cd	0.692 ± 0.007d	29.104 ± 0.061a
S4	0.025 ± 0.002bc	0.685 ± 0.003d	20.910 ± 0.043c
S5	0.018 ± 0.002de	0.725 ± 0.004c	20.554 ± 0.143c
S6	0.012 ± 0.001f	0.814 ± 0.007a	20.623 ± 0.357c
S7	0.017 ± 0.002e	0.507 ± 0.010f	25.323 ± 0.195b

3.5. Effect of inoculation with different growth-promoting actinomycetes on the activities of protective enzymes and related gene expressions in the leaves of *F. taipaiensis* P. Y. Li

3.5.1. Effect of inoculation with different growth-promoting actinomycetes on POD activity and relative gene expression in the leaves of *F. taipaiensis*

In assessing the POD activity within the leaves of *F. taipaiensis*, all treated groups displayed an augmented activity compared to the uninoculated CK group, and the differences with the CK group were statistically significant($P < 0.05$), emphasizing that the inoculation of growth-promoting actinomycetes had a greater effect on the SOD activity in the leaves of *F. taipaiensis*. Notably, the treatment groups S5, S7 and S3 demonstrated activity levels were significantly higher than the CK group, which were 3.14, 2.89 and 2.75 times, respectively. Within the single actinomycetes inoculation groups (S1∼S3), the POD activity followed the sequence: S3 > S2 > S1. In the combined inoculation groups (S4∼S6) encompassing two actinomycetes species, the POD activity hierarchy emerged as S5>S6>S4.

As shown in Fig. 1, the results of analyzing the relative expression of POD genes showed that the relative expression of POD genes in all treatment groups increased to different degrees compared with that in the CK group($P < 0.05$). Specifically, the S5, S7 and S3 groups were significantly higher than that in the CK group, which were 7.97, 6.33 and 4.89 times higher than that in the CK group, respectively. This suggests that the inoculation of growth-promoting actinomycetes increased the activity of POD and the relative expression of genes in the leaves of *F. taipaiensis*.

3.5.2. Effect of inoculation with different growth-promoting actinomycetes on SOD activity and relative gene expression in the leaves of *F. taipaiensis*

The determination of SOD activity in the leaves of *F. taipaiensis* showed that the SOD activity increased to different degrees in all treatment groups compared to the CK group, as illustrated in Fig. 2. The highest SOD activity was observed in the S5 group, which was 3.98 times higher than that in the CK group, followed by S3 group. It indicates that single inoculated *S. zaomyceticus* and mixed inoculated *S. lavendulae* and *S. zaomyceticus*, significantly increased the POD activity in the leaves of *F. taipaiensis*. The differences in the contents of S4, S6 and S7 group, were not statistically significant, indicating that the effects of the three treatments were approximately comparable.

Analysis of relative SOD gene expression revealed a significant increase in the relative SOD gene expression in the leaves of *F. taipaiensis* in all treatments. S5 group had the highest relative gene expression, which was 5.03 times that of the CK group. The S3 group trailed closely, displaying a 4.23-fold rise compared to the CK group. On the whole, these findings denote that inoculating *F. taipaiensis* leaves with growth-promoting actinomycetes instigates discernible variations in both POD activity and the ensuing gene expressions.

3.5.3. Effect of inoculation with different growth-promoting actinomycetes on CAT activity and relative gene expression in the leaves of *F. taipaiensis*

The CAT activity in *F. taipaiensis* leaves was higher than that of the CK group in all treatment groups(Fig. 3). The highest activity was found in the S5 group, being 1.36 times as high as in the CK group, and the differences with other groups were statistically significant ($P < 0.05$). The differences between all treatment groups and CK group were statistically significant ($P < 0.05$), which indicated that the inoculation of different growth-promoting actinomycetes would have a different effect on the CAT activity in the leaves of *F. taipaiensis*.

The relative expression of CAT genes showed that the relative expression of CAT genes in the leaves of *F. taipaiensis* treated with different growth-promoting actinomycetes was increased in comparison with the CK group, and the differences were statistically

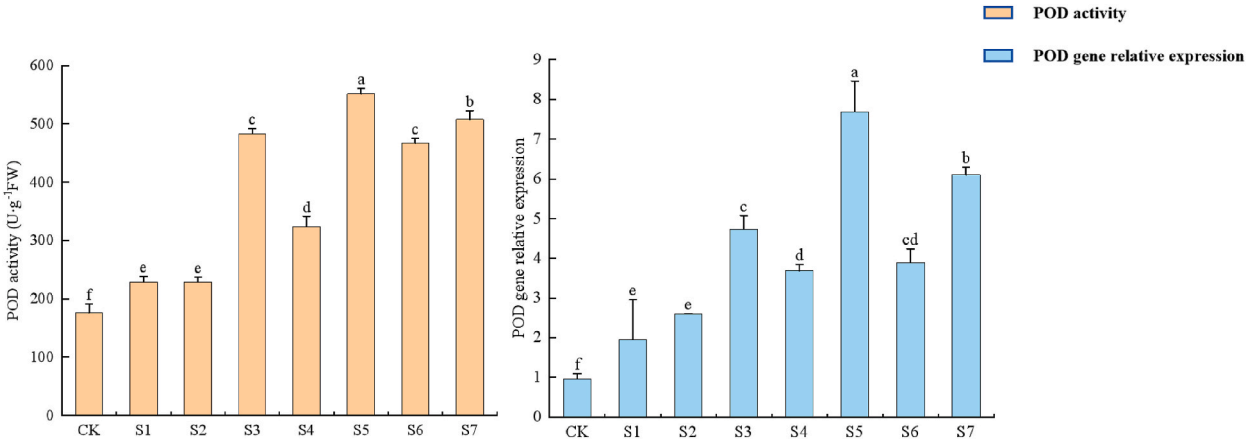

Fig. 1. Effect of inoculation with different growth-promoting actinomycetes on POD activity and relative gene expression in the leaves of *F. taipaiensis* ($\overline{x} \pm s$, n = 3) Different letters in columus indicate statistically significant differences($P < 0.05$).

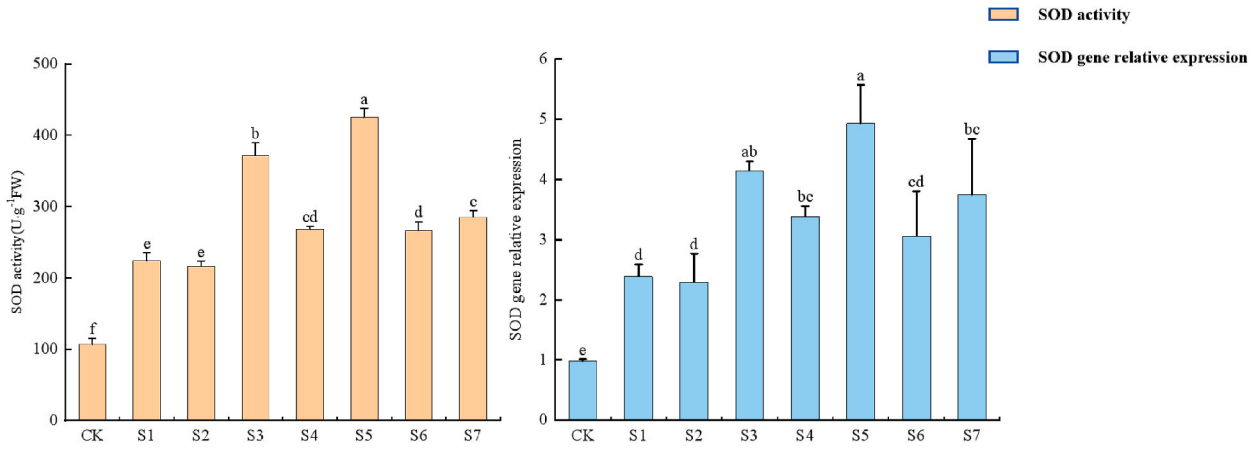

Fig. 2. Effect of inoculation with different growth-promoting actinomycetes on SOD activity and relative gene expression in the leaves of *F. taipaiensis* ($\overline{x}$ ±s, n = 3) Different letters in columus indicate statistically significant differences(P < 0.05).

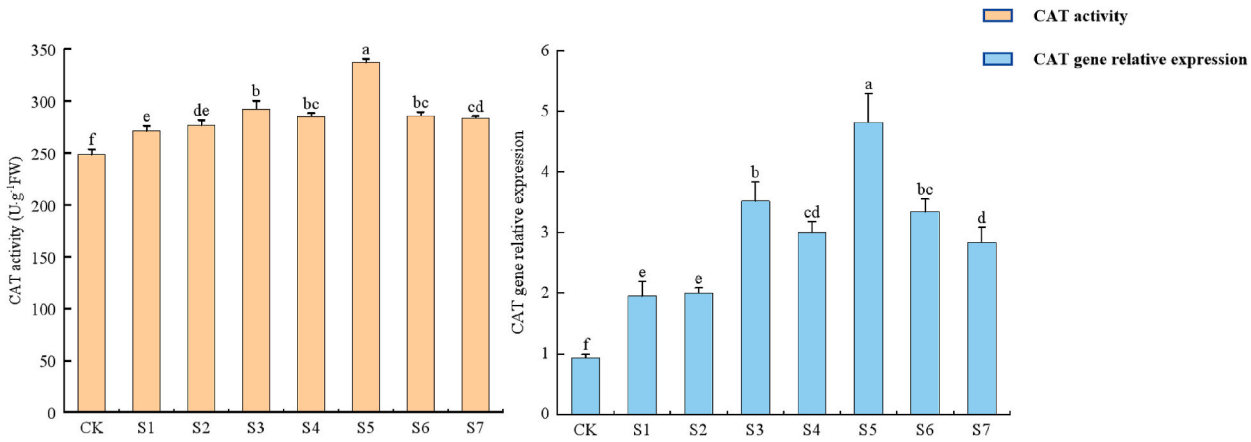

Fig. 3. Effect of inoculation with different growth-promoting actinomycetes on CAT activity and relative gene expression in the leaves of *F. taipaiensis* ($\overline{x}$ ±s, n = 3) Different letters in columus indicate statistically significant differences(P < 0.05).

significant ($P < 0.05$). In this context, the S5 group had the highest relative gene expression, manifesting a substantial 5.16-fold rise compared to the CK group. Consequently, the activity of CAT and the relative gene expression in the leaves of *F. taipaiensis* can be increased to different extents by the inoculation of growth-promoting actinomycetes.

3.6. Correlation analysis of physiological and biochemical indicators leaves of F. taipaiensis

The physiological and biochemical indicators of the leaves of *F. taipaiensis* under different growth-promoting actinomycetes

Table 6
Correlation analysis of physiological and biochemical indicators leaves of *F. taipaiensis*.

Indicators	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	MDA	Soluble Protein	Soluble Sugar	POD	SOD	CAT
Chlorophyll *a*	1								
Chlorophyll *b*	0.574**	1							
Carotenoids	0.765**	0.159	1						
MDA	−0.254	−0.228	−0.361	1					
Soluble Protein	0.254	0.389	0.299	−0.633**	1				
Soluble Sugar	−0.232	−0.326	−0.153	−0.212	0.091	1			
POD	0.339	0.556**	0.248	−0.803**	0.743**	0.004	1		
SOD	0.513*	0.594**	0.286	−0.616**	0.777**	0.202	0.858**	1	
CAT	0.640**	0.607**	0.381	−0.571**	0.539**	0.256	0.774**	0.909**	1

"*" indicates a significant correlation between the two ($P < 0.05$). "**" indicates a very close correlation between the two ($P < 0.01$), N = 3.

treatments were correlated, the results are shown in Table 6. With regard to chlorophyll in the leaves of *F. taipaiensis*, the content of chlorophyll *a* showed a significant positive correlation with chlorophyll *b*, carotenoids, SOD and CAT activities ($P < 0.05$). on the other hand, chlorophyll *b* showed a highly significant positive correlation with three antioxidant enzyme activities ($P < 0.01$). The content of MDA showed a highly significant negative correlation with soluble proteins and the activities of the three antioxidant enzymes ($P < 0.01$), while soluble proteins showed a highly significant positive ccorrelation with the activities of the three antioxidant enzymes ($P < 0.01$). Furthermore, A salient observation was the profound positive correlation amongst the triad of antioxidant enzymes ($P < 0.01$). In stark contrast, carotenoids and soluble sugars did not show any significant correlation with the other indicators.

4. Discussion

Actinomycetes are a class of Gram-positive, high (G + C) bacteria (>55 %) that are widespread in nature. Notably, the secondary metabolites produced during the growth of the actinomycetes mycelium have the advantages of killing other microorganisms, promoting plant growth and development, enhancing soil ecosystems, and increasing crop quality and yield without detriment to the environment [24–27]. The empirical evidence from our study illuminated that all seven treatment groups were significantly higher in leaf growth indicators of *F. taipaiensis* than the CK group, with the S5 group showcasing superior outcomes. This can be attributed to the intrinsic capability of actinomycetes to synthesize pro-biotic entities such as cytokinins and growth factors, thereby indirectly bolstering plant growth.

Leaves, being pivotal photosynthetic organs in plants, play a cardinal role in their growth [28]. Plant growth and leaf area are closely related, the latter not only governs photosynthate accrual but also mirrors plant growth, acting as a crucial determinant of ecosystem dynamics, energy transduction, and overall plant productivity [29]. The study investigations revealed a conspicuous enhancement in the leaf area across all treatment groups in contrast to the non-inoculated CK group. The largest leaf area was in the S5 group, indicating that inoculation with the growth-promoting actinomycetes accelerated the growth rate of the leaves of *F. taipaiensis.*

Photosynthetic parameters may affect the level of photosynthetic capacity of plants, requiring the harmonized interplay of factors such as net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intracellular CO_2 concentration (Ci) [28]. Our findings corroborate that actinomycete inoculation elevated the Pn, Tr, Gs and Ci relative to the CK group. It is presumed that the inoculation of growth-promoting actinomycetes promoted the uptake of nutrient elements, enhanced photosynthesis, and facilitated the utilisation of nutrients and light by the plants in *F. taipaiensis*. It is well known that chlorophyll and carotenoids play a very important role in the photosynthesis process of plants. Chlorophyll is the material basis of plant photosynthesis and the main pigment, and its content directly affects the strength of plant photosynthesis and material synthesis [30,31]. Carotenoids are natural water-soluble scavengers of free radicals, which play an important role in the absorption of light energy, protection of chlorophyll, and bursting of reactive oxygen species [32]. In this study, the photosynthetic pigments in the leaves of *F. taipaiensis* were increased to different degrees in all treatment groups compared with those in CK group. The highest content of light chlorophyll *a* and chlorophyll *b* was found in S5 group and the highest content of carotenoids was found in S7 group. Hence, the inoculation of growth-promoting actinomycetes can increase the content of photosynthetic pigments and enhance the photosynthesis of *F. taipaiensis*, which can ultimately achieve the purpose of promoting plant growth.

When plant cells are subjected to adversity stress, membrane lipid peroxidation occurs, which can produce a large amount of malondialdehyde (MDA). Consequently, MDA content is often used as one of the indicators of membrane lipid peroxidation. Soluble sugars and soluble proteins, as cellular osmoregulators, accumulate in large quantities in cells when the plant body is exposed to adversity in order to improve water retention and plant cell resistance [33]. In the present study, the contents of soluble sugars and soluble proteins in the leaves of *F. taipaiensis* were affected to different extents by inoculation with growth-promoting actinomycetes. The content of soluble sugars increased in the treatment groups with the exception of the S7 group, and the content of MDA generally showed a decreasing trend.

The POD, SOD and CAT are the main enzymes in the plant's antioxidant system and their activity levels respond to the plant's exposure to external stresses. The antioxidant enzyme SOD catalyses the disproportionation of superoxide anion ($O_2^{\cdot-}$) to form H_2O_2 and O_2, while POD and CAT promote the reduction of H_2O_2 to H_2O and O_2 [34]. After inoculation with actinomycetes, a discernible escalation in the activities ofthree protective enzymes was observed in *F. taipaiensis* leaves. Collectively, S5 group had the highest activity. In addition, this study found that compared with the CK group, the inoculation of growth-promoting actinomycetes could increase the expression of antioxidant enzyme-related genes in the leaves of *F. taipaiensis* to varying degrees, among which the expression of related genes was highest in the S5 group, which was consistent with the trend of changes in the activities of antioxidant enzymes(SOD, POD and CAT) and indicated that the level of enzyme activity was affected by the expression of related genes. The SOD, POD and CAT, as the main antioxidant enzymes in plants, increasing the expression of RNAs that regulate their protein translation directly affects the activity of the corresponding enzymes, improving the ability of *F. taipaiensis* to survive in harsh environments.

5. Conclusion

In summary, the inoculation of single strains or combinations of diverse growth-promoting actinomycetes can promote the growth of *F. taipaiensis*, increase the content of photosynthetic pigments in the leaves, reduce the content of MDA, enhance the activities of SOD, POD and CAT enzymes and the expression of related genes in *F. taipaiensis* leaves, and promote the accumulation of osmoregulatory substances such as soluble sugars and soluble proteins, which are of great importance to the growth and development of *F. taipaiensis*. It can promote the growth and development of *F. taipaiensis*, enhance photosynthesis of *F. taipaiensis* leaves and improve the resistance to environmental stress. At present, the study on the interaction between actinomycetes and rhizosphere

microorganisms has become an important way to explain the vegetative growth of *F. taipaiensis*. In order to further clarify the gain effect of actinomycetes on the growth of *F. taipaiensis*, it is necessary to further explore the growth-promoting mechanism of actinomycetes on the growth of *F. taipaiensis*, so as to provide reference for standardized planting and quality improvement of *F. taipaiensis*.

Funding

This study is funded by the Yunnan Academician (Expert) Workstation Project: Xiaobo Expert Workstation in Yunnan Province (202105AF150073), Key R&D project of Yunnan Province(202102AA310031), Key project of Natural Science Foundation of Chongqing Municipal Education Commission (KJZD-K202101201) and Chongqing Wanzhou District Science and Technology Innovation Project(wzstc20210216).

CRediT authorship contribution statement

Xiaotian Kong: Writing – original draft, Investigation, Conceptualization. **Liang Han:** Methodology. **Liqin Yang:** Methodology. **Zhifen Shi:** Investigation. **Jiaqi Lang:** Investigation. **Mingyan Ye:** Investigation. **Bo Xiao:** Writing – original draft. **Xubing Chen:** Writing – review & editing. **Nong Zhou:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] National Pharmacopoeia Commission, Pharmacopoeia of the people's Republic of China: part I, Chin. Med. Sci. Tech. Press (2020) 38–39.
[2] Q. Tian, Y. Qiang, K.K. Chen, Z.Z. Wang, Advance of valuable endangered medicinal plant *F. taipaiensis*, Shaanxi J. Agric. Sci. 64 (9) (2018) 96–98.
[3] X.F. Cong, H. Chen, W.M. Li, D. Li, S.F. Li, Habitat investigation and rhizosphere soil analysis of wild *Fritillaria taibaiensis*, J. Agric. For. 12 (3) (2022) 55–58.
[4] R. Kamaei, F. Faramarzi, M. Parsa, M. Jahan, The effects of biological, chemical, and organic fertilizers application on root growth features and grain yield of *Sorghum*, J. Plant Nutr. 42 (18) (2019) 2221–2233.
[5] D. Kour, K.L. Rana, N. Yadav, A.N. Yadav, A. Kumar, Rhizospheric Microbiomes:biodiversity, Mechanisms of Plant Growth Promotion, and Biotechnological Applications for Sustainable Agriculture, Springer Singapore press, 2019.
[6] O.S. Olanrewaju, O.O. Babalola, *Streptomyces*: implications and interactions in plant growth promotion, Appl. Microbiol. Biotechnol. 103 (3) (2019) 1179–1188.
[7] K.R.K. Reddy, G. Jyothi, C. Sowjanya, K. Kusumanjali, N. Malathi, K.R.N. Reddy, Plant Growth-Promoting Actinomycetes: Mass Production, Delivery Systems, and Commercialization, Springer Singapore press, 2016.
[8] A.P. Chakraborty, P. Ray, U. Chakraborty, B. Chakraborty, Actinomycetes as growth promoters and bioprotectors for plant health improvement, J. Mycopathol. Res. 61 (2) (2023) 157–170.
[9] X.J. Zhou, Y.X. Yang, P. Hu, M. Zhang, Y.L. Xia, Investigation on the resources of *Fritillaria taipaiensis*, J. Anhui Agric. Sci. 43 (17) (2015) 84–85.
[10] S. Fu, H. Chen, D. Yuan, H. Jia, C. Pu, Q. Lu, Investigation of chongqing *Fritillaria taipaiensis* resources, Chin. J. Inf. Tradit. Chin. Med. 23 (9) (2016) 1–4.
[11] P. Hu, Y.L. Xia, Y.X. Yang, P. Wu, T.Z. Chen, X.J. Zhou, Investigation on the resources of *Fritillaria taipaiensis* in wanyuan calyx mountain, J. Anhui Agric. Sci. 45 (15) (2017) 9–10, 17.
[12] P. Ma, L. Wang, Y.M. Wang, R. Peng, Effects of Taibai fritillaria for relieving cough,expelling phlegm, anti-inflammatory, Pharmacol. Clin. Chin. Mater. Med. 30 (1) (2014) 87–89.
[13] Z. Xiao, F. Mou, J. Deng, P. Hao, J. Wang, Z. Xie, Inhibitory effects and its possible mechanisms of total alkaloids extracted from *Fritillaria Taipaiensis* on the proliferation of human colon adenocarcinoma cell line SW480 in vitro, Anti-Tumor Pharm 8 (4) (2018) 505–511.
[14] H. Yao, C.Y. Jiang, Y.H. Wang, Y. Luo, J. Tao, D.-Q. Guo, N. Zhou, Research on Extraction Process and Chemical Composition of Volatile Oil of *Fritillaria Taipaiensis* P.Y. Li, Food Ferment. Ind., 2024, pp. 1–9.
[15] C.M. Mei, F.G. Chen, Y.W. Zhao, D. Wang, C.C. Shi, H.K. Qiu, N. Zhou, W.D. Li, Compositional analysis of 11 nucleosides and bases in *Fritillaria taipaiensis* P. Y. Li from different origins and the differences in their origin, Tradit. Chin. Drug Res. Clin. Pharmacol. 35 (3) (2024) 411–418.
[16] M.N. Hou, Z.P. Liu, S.P. Hou, et al., Comparison of polysaccharide content and antioxidant activity of *Fritillaria taipaiensis* P. Y. Li in different rears, Chin. Wild Plant Resour. 42 (8) (2023) 44–47, 80.
[17] M.-J. Mu, D.-G. Zhang, H. Zhang, M. Yang, D.-Q. Guo, N. Zhou, Correlation between rhizospheric microorganisms distribution and al;kaloid content of *Fritillaria taipaiensis*, China J, Chin. Mater. Med. 44 (11) (2019) 2231–2235.
[18] J. Zhang, B. Feng, C. Wu, Effects of arbuscular mycorrhizal fungi on growth and Q-Markers of *Fritillaria Taipaiensis*, Chin. J. Inf. Tradit. Chin. Med. 27 (7) (2020) 88–93.
[19] Z. Shi, F. Pan, X. Kong, J. Lang, M. Ye, Q. Wu, G. Wang, L. Han, N. Zhou, Effects of inoculation with phosphate solubilizing bacteria on the physiology, biochemistry, and expression of genes related to the protective enzyme system of *Fritillaria taipaiensis* P. Y. Li, Phyton-Int. J. Exp. Bot. 93 (2) (2024) 247–260.
[20] Z. Shu, X.S. Zhang, J. Chen, G.Y. Chen, D.Q. Xu, The simplification of chlorophyll content measurement, Plant Physiol. J. 46 (4) (2010) 399–402.
[21] Q.E. Xiong, Plant Physiology Experiment Tutorial, Sichuan Sci. Technol. Publ. House press, Chengdu, 2003.
[22] C.N. Giannopolitis, S.K. Ries, I. Superoxidedismutases, Occurrence in high plants, Plant Physiol. 59 (2) (1977) 309–314.
[23] J.F. Gao, Plant Physiology Experiment Guidance, Higher Education press, Beijing, 2006.
[24] Y.Q. Guan, B.J. Zhang, J.C. Han, H.P. Liu, Research progress on the application of plant endophytic actinomycetes in agriculture, Agric. Technol. Equip. (6) (2011) 52–56.
[25] J. Yang, M.D. Liao, Actinomycetes resources and its application, World Pestic 36 (1) (2014) 22–26.
[26] Y. Yang, K.-T. Li, The overview of actinomycetes resources and its active substances, Biol. Disaster Sci. 42 (1) (2019) 7–14.
[27] Y.L. Song, J. Yu, S.G. Chen, et al., Effects of complex microbial agent on cotton physiological characteristics, microorganism and physicochemical properties in rhizosphere Soil, Soils 51 (3) (2019) 477–487.
[28] Z. Li, L. Luo, J. Lang, M. Ye, F. Yin, J. Zhao, N. Zhou, Effect of organophosphate-solubilizing bacteria on photosynthesis, physiology, and biochemistry of *Paris polyphylla* var. *Yunnanensis*, Chin. J. Exp. Tradit. Med. Formulae 28 (16) (2022) 165–171.
[29] N.H. Broge, J.V. Mortensen, Deriving green crop area index and canopy chlorophyll density of winter wheat from spectral reflectance data, Remote Sens. Environ. 81 (1) (2002) 45–57.
[30] X. Zhao, L. Ye, Effects of salt and alkali stress on the growth, quality and photosynthetic characteristics of Alfalfa, Jiangsu Agric. Sci. 45 (21) (2017) 176–180.
[31] J. Zhang, Y. Guan, L. Si, X. Peng, J. Zhou, Effects of Pb^{2+} and Cd^{2+} combined stress on photosynthesis of *Morus alba*, J. Beijing For. Univ. 40 (4) (2018) 16–23.

[32] Y. Xu, C. Hu, W. Zhang, L. Liu, R. Zhao, Effect of simulated drought stress on photosynthesis related indexes at seedling stage of wild soybeans, Soybean Sci 32 (3) (2013) 341–344.
[33] J. Yang, Y.M. Jiang, F. Zhou, J. Zhang, H.D. Luo, S.J. Tian, Effects of PEG simulated drought stress on seedling morphology and physiological characteristics of different drought-resistance Maize varieties, Crop. (1) (2021) 82–89.
[34] X.Y. Zhang, Z.B. Ye, Y.Y. Zhang, Advances in physiological and molecular mechanism of plant response to cadmium stress, Plant Physiol. J. 57 (7) (2021) 1437–1450.