



ARTICLE

Effects of *Piriformospora indica* on the Respiration of *Taxus chinensis* var. *mairei* under Water Stress

Longsheng Hu, Xinru He, Pujie Wei, Bing Sun, Yongjun Fei and Die Hu*

College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025, China

*Corresponding Author: Die Hu. Email: hudie.16@163.com

Received: 20 January 2021 Accepted: 31 March 2021

ABSTRACT

Seedlings of *Taxus chinensis* var. *mairei* were used as experimental materials to study the adaptation of *Piriformospora indica* to this plant under water stress. The materials were divided into two groups, namely, with or without inoculation with *P. indica*. Each group was subjected to four different levels of water stress. Vitality and physiological and biochemical indexes of the roots of *T. chinensis* var. *mairei* were regularly measured. Under water stress, *T. chinensis* var. *mairei* had significantly decreased root vitality; root vitality was higher in inoculated roots than in uninoculated roots. Under intense water stress, the inoculated roots had a higher soluble sugar content than the uninoculated roots. Under water stress, *T. chinensis* var. *mairei* experienced decreased activity of aerobic respiratory metabolic enzymes. The activity of anaerobic respiratory metabolic enzymes and alcohol dehydrogenase initially increased and then decreased, whereas that of lactate dehydrogenase increased. The inoculated roots had a higher activity of respiratory metabolic enzymes than the uninoculated roots. As water stress was further intensified, the roots had significantly decreased activity of aerobic respiratory metabolic enzymes and significantly increased activity of anaerobic respiratory metabolic enzymes. The activity of respiratory metabolic enzymes decreased faster in the uninoculated roots than in the inoculated roots. This study demonstrated that *Piriformospora indica* plays a positive role in enhancing the antihypoxic ability of *T. chinensis* var. *mairei*, thereby alleviating plant damage due to water stress.

KEYWORDS

Taxus chinensis var. *mairei*; *Piriformospora indica*; water stress; respiration

1 Introduction

As a relict tree species of the Tertiary Period, *Taxus chinensis* var. *mairei* is listed as a “the state-protected one-grade rare and endangered plants” [1,2]. This tree is an important novel natural anticancer drug with a special anticancer mechanism and good anticancer effects [3]. Taxol is mainly derived from plant resources of *Taxus* Linn [4]. Owing to its rarity and medicinal value, wild resources of *Taxus* plants have been severely exploited. In addition, *T. chinensis* var. *mairei* can purify the air by absorbing harmful substances. Thus, this species plays a role in the sterilization, disinfection, and protection of the ecological environment [5]. However, *T. chinensis* var. *mairei* is difficult to sow because it has a low seedling survival rate [6,7], and it is intolerant to flooding. Thus, its survival situation is relatively grim.



Colonization of plants by *Piriformospora indica* can make them resistant to biotic and abiotic stresses [8], increase plant biomass [9], and increase the formation of lateral roots and root hairs [10]. Colonized plant roots are more developed than uncolonized roots, and the survival rate is high [11,12]. Water stress is an important factor affecting plant growth [13]. It has a direct impact on roots. Thus, plant roots can act as an important sign of response to waterlogging stress [14–17]. When a tree species with a weak water tolerance is subjected to water stress, its photosynthesis weakens [18], and its growth and development are inhibited [19], causing root death and plant wilting [20,21].

In this study, *P. indica* was inoculated into the roots of *T. chinensis* var. *mairei* under potted conditions to study the adaptation of this tree species to water stress. This study aimed to provide a theoretical basis and scientific methods for cultivating *T. chinensis* var. *mairei* against stress. This work also aimed to increase the stress resistance of *T. chinensis* var. *mairei* in southern areas with a high water content [1].

2 Materials and Methods

2.1 Overview of the Test Site

This experiment was conducted in Yangtze University. Jingzhou City (111°15′–114° 05′E, N29° 26′–31° 37′) is located in the central and southern parts of Hubei Province. Areas with a subtropical monsoon climate such as Jingzhou City has four distinct seasons, sufficient sunshine, and abundant precipitation. This city has a total solar radiation of about 104–110 kcal/cm², an average annual sunshine hours of 1800–2000 h, an annual average temperature of 15.9°C–16.6°C, an annual rainfall of 1100–1300 mm, and an annual frost-free period of 250–267 d. The experimental seedlings were placed outdoors. In a previous study [22], we established that *T. chinensis* var. *mairei* can be grown in Jingzhou City.

2.2 Experimental Design

In March 2019, seedlings of *T. chinensis* var. *mairei* with good growth and consistent growth vigor were transplanted in pots and placed in a garden to allow natural growth. The substrate consisted of a mixture of nutrient soil, river sand, and perlite at a ratio of 2:1:1. After 3 months of routine management, the treatment was commenced in June. First, 100 mL of *P. indica* diluent was cultured in potato dextrose agar medium (200 g of potato, 20 g of glucose, 15–20 g of agar, and 1000 mL distilled water) for 5 d and then in potato dextrose liquid medium (11 g of potato infusion, 20 g of dextrose, 15 g of agar, and 1000 mL distilled water) for 1 week. This culture was added to each pot in the *T. chinensis* var. *mairei* treatment group. The same amount of deionized water was added into each pot in the control group. Forty days after the inoculation, the root tips of *T. chinensis* var. *mairei* were cleaned and cut into sections of about 1 cm. The root tips were soaked in 10% KOH for almost 12 h. Afterward, the root tips were rinsed with deionized water for 6–9 times, placed on glass slides, and then dropped with an appropriate amount of trypan blue for about 12 min to observe the plant tissues under a stereomicroscope.

The materials were divided into two groups. One group was inoculated with *P. indica*, whereas the other group was not inoculated. Each group of test materials was subjected to four different degrees of water stress: each group was watered up to 0, 1/2, 1/3, and 1/4 of the height of the pot, and the water level was maintained at these levels; these groups were labelled as Ckn, N2, N3, and N4 for the uninoculated seedlings, respectively, and Cky, Y2, Y3, and Y4 in the inoculated seedlings, respectively (Tab. 1). All pots were 10.6 cm in height, with an outer diameter of 11.9 cm and an inner diameter of 11.4 cm. Two groups of different water stresses were repeated for each treatment with 20 plants, for a total of 160 plants. After 40 d of inoculation, samples were collected every 4 days for a total of five times. The physiological and biochemical indexes of the underground roots of *T. chinensis* var. *mairei* were measured each time.

Table 1: Experimental design

Treatment	Normal	Watering up to 1/4 of the height of the pot	Watering up to 1/3 of the height of the pot	Watering up to 1/2 of the height of the pot
Inoculation with <i>Piriformospora indica</i>	CKy	Y4	Y3	Y2
Without inoculation with <i>Piriformospora indica</i>	CKn	N4	N3	N2

2.3 Determination of Test Indexes

Root vitality was measured via the triphenyl tetrazolium chloride method [23]. Soluble sugar content was determined by anthrone colorimetry [24]. Malic acid content was quantified by NaOH titration [25]. Malate dehydrogenase (MDH) activity was assessed following a previously described method [26]. Activities of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and alcohol dehydrogenase (ADH) were evaluated using the lactic acid kit of Nanjing Jiancheng Bioengineering Institute. Lactic acid content was measured using the same kit.

2.4 Statistical Analysis

Data were analyzed by one-way ANOVA with Duncan's multiple range tests to separate means by using the program SAS 10.0 (SAS Institute, Inc., Cary, NC). Correlation analysis was conducted using SPSS 18.0. Different letters in the graphs or the tables indicate significant differences at $P < 0.05$.

3 Results and Analysis

3.1 *P. indica* Colonization in the Roots of *T. chinensis* var. *mairei* and Comparison of Plant Growth under Different Water Stresses

The roots of uninoculated *T. chinensis* var. *mairei* had no chlamydo spores within the roots' cells (Fig. 1b). By contrast, 40 d after the inoculation, chlamydo spores were observed within the roots' cells of inoculated plants (Fig. 1a). The chlamydo spores of *P. indica* were arranged in clusters in the roots.

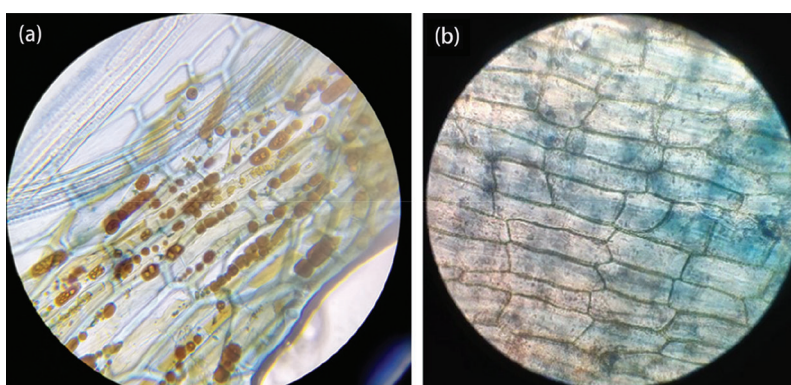


Figure 1: Comparison of colonization of *Piriformospora indica* in the roots of *Taxus chinensis* var. *mairei*
 Note: ^aStand for chlamydo spores stained in the roots of *T. chinensis* var. *mairei*. ^bStand for uninoculated root cells stained in the roots of *T. chinensis* var. *mairei*. The samples were observed at 400× magnification

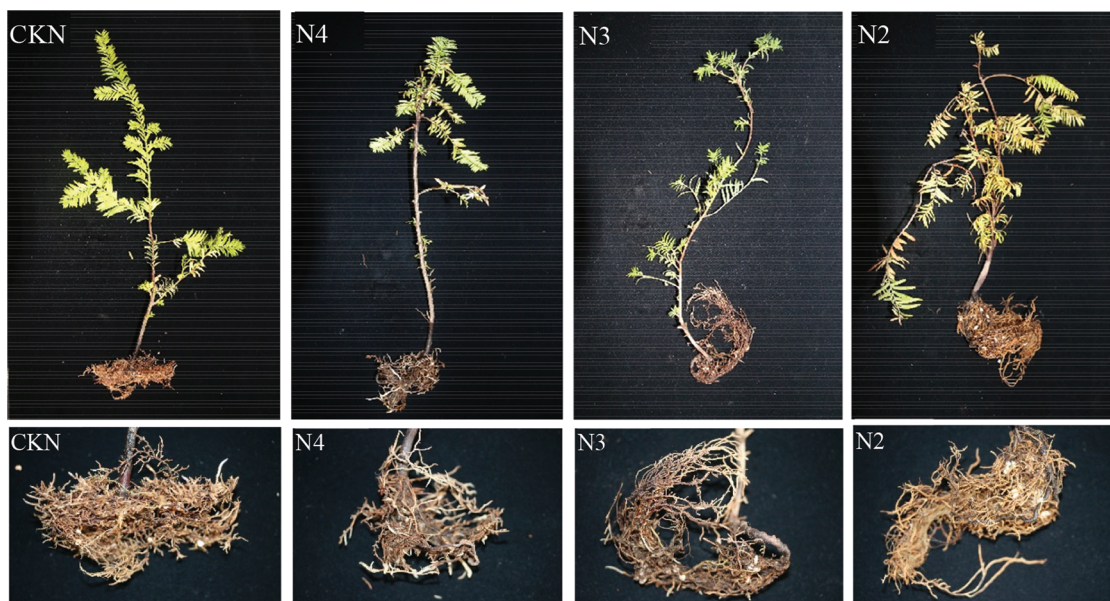


Figure 2: Growth of *Taxus chinensis* var. *mairei* seedlings and their roots under different water stresses on the 16th day

On the 16th day, the growth of uninoculated *T. chinensis* var. *mairei* seedlings and their roots gradually deteriorated as water stress intensified (Fig. 2). The growth of CKN seedlings was the best; they had luxuriant leaves, well-developed root systems, and more new roots. N4 seedlings vigorously grew, with better growth and new roots in their root systems. N3 seedlings were curved and thin, with sparse roots and few new roots. N2 seedlings had the worst growth; they were thin and weak, with yellow leaves, wilted roots, and no new roots.

3.2 Effects of *P. indica* on the Root Vitality of *T. chinensis* var. *mairei* under Water Stress

On the same day, the root vitality of Y4, Y3, Y2, N4, N3, and N2 seedlings decreased as water stress intensified (Fig. 3). On the 4th day, the difference in root vitality between CKn, Y4, Y3, and N4 seedlings was not significant ($P > 0.05$). However, on the 8th day, the difference in root vitality between CKn, N4, N4, and N2 seedlings was significant ($P < 0.05$). However, on the 12th day, the difference in root vitality between CKy, Y4, Y3, and Y2 seedlings was significant ($P < 0.05$). Moreover, root vigor index was higher in the inoculated roots than in the uninoculated roots. Results demonstrated that *T. chinensis* var. *mairei* experienced low root vitality as water stress intensified. Inoculated *T. chinensis* var. *mairei* had a higher root vitality than the uninoculated seedlings. Moreover, the root vitality of the uninoculated *T. chinensis* var. *mairei* decreased much faster and was substantially lower than that of inoculated seedlings.

3.3 Effects of *P. indica* on the Soluble Sugar Content in the Roots of *T. chinensis* var. *mairei* under Water Stress

As water stress intensified, the content of soluble sugars in the roots of *T. chinensis* var. *mairei* increased. The content initially increased and then decreased (Fig. 4). On the 4th day of inoculation, the differences between CKy, Y4, Y3, and Y2 were significant ($P < 0.05$). Similarly, on the 16th day of inoculation, the differences between CKy and Y3, Y3, and Y4 were significant ($P < 0.05$); however, the differences

between Y2, Y3, and Y4 were not significant ($P > 0.05$). Results showed that soluble sugar content was higher in the inoculated group than in the uninoculated group. On the 4th day, the differences between N4, N3, and CKn were significant ($P < 0.05$). On the 16th day, the differences between CKn and N2 were significant ($P < 0.05$), but those between N3, N2, and N4 were not significant ($P > 0.05$). The soluble sugar content of each treatment group was lower than that of the control group. Inoculated *T. chinensis* var. *mairei* seedlings had a higher soluble sugar content in the roots than the uninoculated seedlings; the reduction in soluble sugar content was slower in the former than that in the latter.

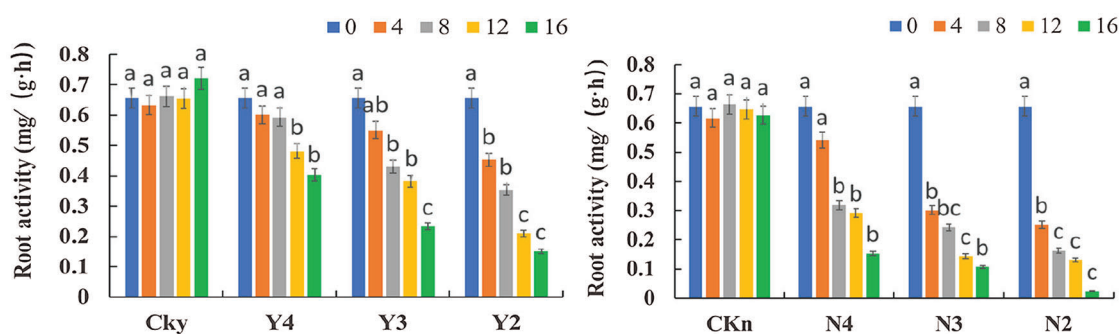


Figure 3: Root activity in the inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 day of watering

Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group in the figure indicate significant differences ($P < 0.05$)

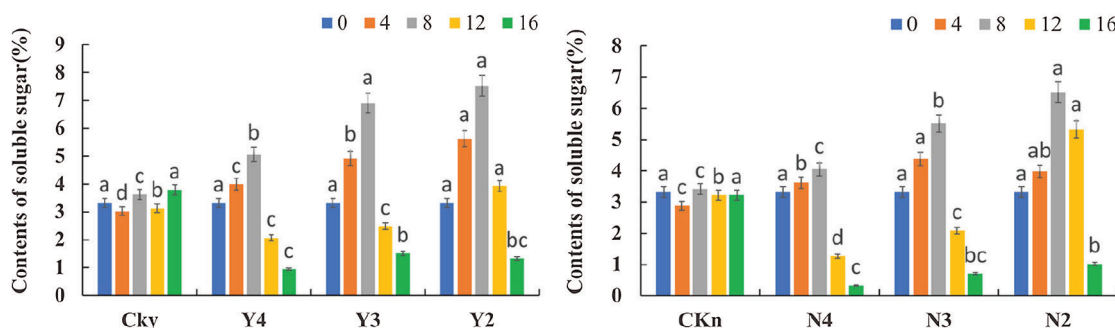


Figure 4: Soluble sugar content in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering

Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group indicate significant differences ($P < 0.05$)

3.4 Effect of *P. indica* on MDH Activity in the Roots of *T. chinensis* var. *mairei* under Water Stress

As water stress intensified over time, MDH activity in the two groups gradually decreased (Fig. 5). On the 4th day of inoculation, the differences between Y4, Y3, Y2, and CKy were significant ($P < 0.05$). On the 16th day of inoculation, the difference between Y3 and Y4 was not significant ($P > 0.05$), whereas that between Y2, Y3, and CKy was significant ($P < 0.05$). As water stress further intensified, MDH activity in the roots decreased. On the 4th day, the differences between CKn, N4, N2, and N3 were significant ($P < 0.05$). On the 16th day, the differences between the treatment groups significant ($P < 0.05$). Uninoculated

T. chinensis var. *mairei* had a faster and a greater reduction in MDH activity than the inoculated seedlings. The inoculated *T. chinensis* var. *mairei* had a higher MDH activity in the roots than the uninoculated seedlings.

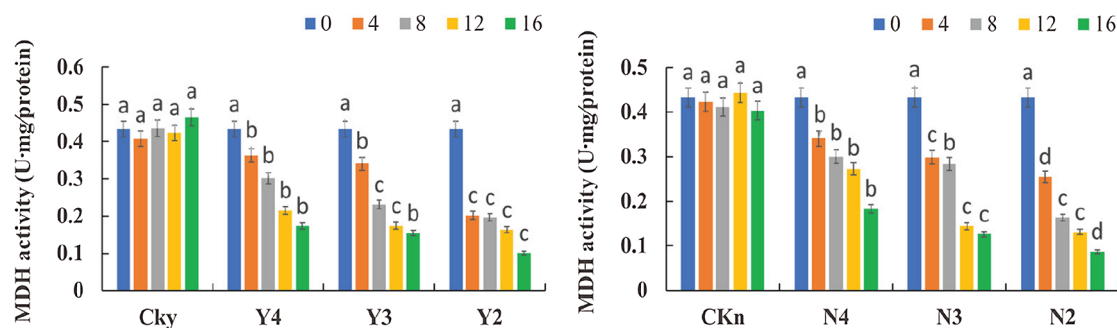


Figure 5: Malate dehydrogenase activity in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering
Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group in the figure indicate significant differences ($P < 0.05$)

3.5 Effects of *P. indica* on SDH Activity in the Roots of *T. chinensis* var. *mairei* under Water Stress

As water stress intensified over time, the activity of MDH in the two groups gradually decreased, but the decrease was slower in the inoculated group than in the uninoculated group (Fig. 6). On the 4th day of inoculation, the differences between CKy, Y3, and Y2 were significant ($P < 0.05$), but the difference between CKy and Y4 was not significant ($P > 0.05$). On the 16th day of inoculation, the differences between the treatments were significant ($P < 0.05$). On the 4th day, the differences between CKn and N2 and N3 were significant ($P < 0.05$) but not with N4 ($P > 0.05$). On the 16th day, the differences between CKn and N4, N2, and N3 were significant ($P < 0.05$). As water stress further intensified, SDH activity in the roots decreased. Inoculated *T. chinensis* var. *mairei* had a higher SDH activity in the roots than the uninoculated seedlings. The uninoculated *T. chinensis* var. *mairei* had a faster and greater reduction in SDH activity in the roots than the inoculated seedlings.

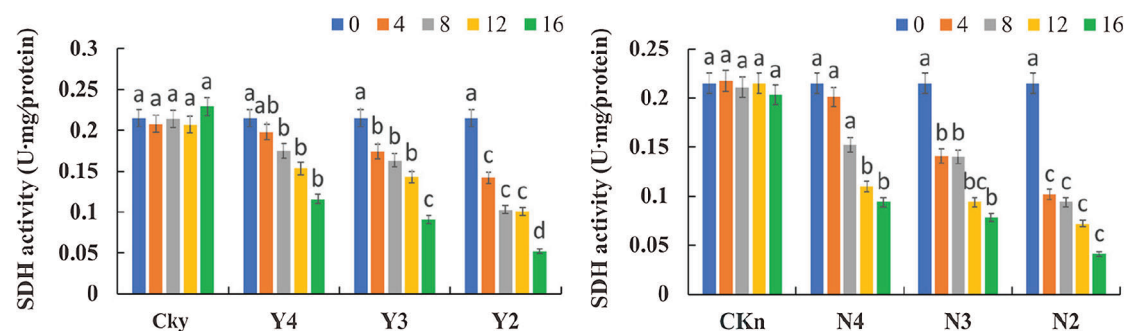


Figure 6: Succinate dehydrogenase activity in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering
Note: Differences between the treatment groups were analyzed with the same number of days. Different letters from the same group indicate significant differences ($P < 0.05$)

3.6 Effects of *P. indica* on the Malic Acid Content in the Roots of *T. chinensis* var. *mairei* under Water Stress

As water stress intensified over time, the malate content in the two groups gradually decreased (Fig. 7). On the 4th day of inoculation, the differences between CKy and Y4, Y3, and Y2 were not significant ($P > 0.05$). On the 12th day, the differences between CKy and Y4, Y3, and Y2 ($P < 0.05$) and between Y3 and Y4 were significant ($P < 0.05$). As water stress further intensified, the malic acid content in the roots decreased. On the 16th day, the differences between CKy and Y4, Y3, and Y2 were significant ($P < 0.05$). On the 4th day, the differences between CKn and N2 and N3 were significant ($P < 0.05$) but not significant between CKn and N4 ($P > 0.05$). On the 8th and 16th days, the differences between the treatments were significant ($P < 0.05$). The uninoculated *T. chinensis* var. *mairei* seedlings had a faster and greater reduction in malic acid content in the roots than the inoculated seedlings. The inoculated *T. chinensis* var. *mairei* seedlings had a higher malic acid content in the roots than the uninoculated seedlings.

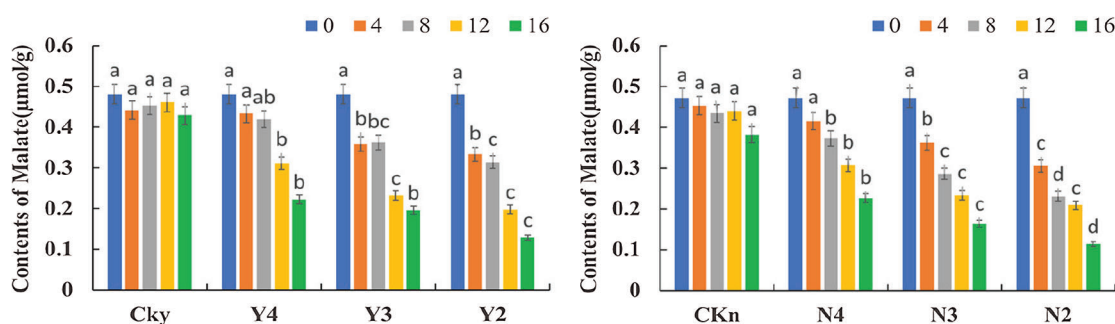


Figure 7: Malate content in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering

Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group indicate significant differences ($P < 0.05$)

3.7 Effect of *P. indica* on LDH Activity in the Roots of *T. chinensis* var. *mairei* under Water Stress

As water stress intensified over time, the activity of LDH in the uninoculated seedlings initially increased and then stabilized; by contrast, in the inoculated group, LDH activity initially increased and then decreased (Fig. 8). On the 4th day of inoculation, the differences between CKy and Y4, Y3, and Y2 were not significant ($P > 0.05$). On the 16th day, the differences between CKy and Y4, Y3, and Y2 and between CKy and Y3 and Y2 were significant ($P < 0.05$). On the 4th day, the differences between CKn and N4 and N3 were not significant ($P > 0.05$), but the difference between CKn and N2 was significant ($P < 0.05$). On the 16th day, the differences between CKn and N4, N3, and N2 were significant ($P < 0.05$), but the difference between N2 and N3 was not significant ($P > 0.05$), with increased LDH activity in the roots. As water stress further intensified over time, the *T. chinensis* var. *mairei* seedlings had increased LDH activity in the roots. The inoculated *T. chinensis* var. *mairei* seedlings had a higher and faster increase in LDH activity in the roots than the uninoculated seedlings. Hence, inoculation with *P. indica* increased the LDH activity in the roots of the *T. chinensis* var. *mairei* seedlings.

3.8 Effects of *P. indica* on ADH Activity in the Roots of *T. chinensis* var. *mairei* under Water Stress

Over time, ADH activity in the two treatment groups initially increased and then decreased (Fig. 9). On the 8th day of inoculation, the differences between CKy and Y4, Y3, and Y2 were significant ($P < 0.05$). On the 16th day, the differences between CKy and Y3 and Y2 were significant ($P < 0.05$), but the difference between Y3 and Y2 was not significant ($P > 0.05$). On the 8th day, the differences between CKn and N4,

N3, and N2 were significant ($P < 0.05$). On the 16th day, the differences between CKn and N3 and N2 were significant ($P < 0.05$), but the difference between N4 and N3 was not significant ($P > 0.05$). Thus, as water stress intensified, ADH activity in the roots of *T. chinensis* var. *mairei* increased. ADH activity in the roots initially increased and then decreased. The inoculated *T. chinensis* var. *mairei* seedlings has a higher and a faster increase in ADH activity in the roots than the uninoculated seedlings. Therefore, inoculation with *P. indica* increased the ADH activity in the roots of *T. chinensis* var. *mairei*.

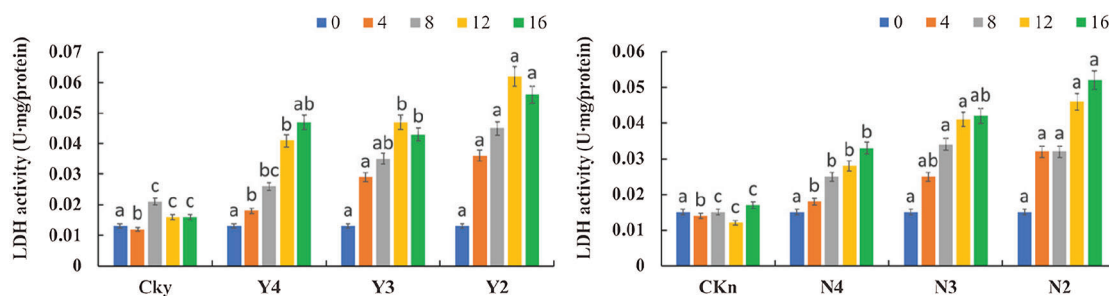


Figure 8: Lactate dehydrogenase activity in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering
Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group indicate significant differences ($P < 0.05$)

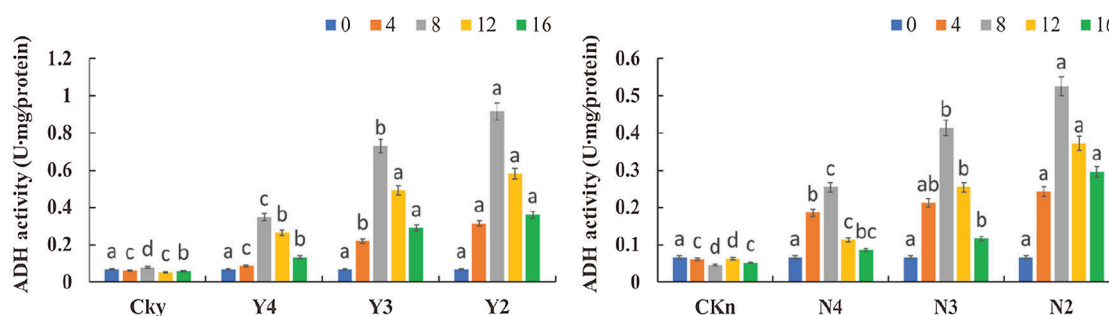


Figure 9: Alcohol dehydrogenase activity in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering
Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group indicate significant differences ($P < 0.05$)

3.9 Effects of *P. indica* on Lactic Acid Content in the Roots of *T. chinensis* var. *mairei* under Water Stress

As water stress intensified over time, the lactic acid content in the two treatment groups initially increased and then decreased (Fig. 10). On the 8th and 12th days of inoculation, the differences between CKy and Y4, Y3, and Y2 were significant ($P < 0.05$). On the 16th day, the differences between CKy and Y4, Y3, and Y2 were significant ($P < 0.05$), but the difference between Y4 and Y3 was not significant ($P > 0.05$). On the 8th day, the differences between CKn and N4, N3, and N2 were significant ($P < 0.05$). On the 16th day, the difference between N4 and N3 was not significant ($P > 0.05$), but the differences between CKn and N4 and N2 were significant ($P < 0.05$). As water stress further intensified over time, lactic acid content initially increased and then decreased. The inoculated *T. chinensis* var. *mairei* seedlings had a higher and faster increase in lactic acid content in the roots than the uninoculated seedlings. The uninoculated *T. chinensis* var. *mairei* seedlings has a greater reduction in lactic acid content than the

inoculated seedlings. Hence, inoculation with *P. indica* increased lactic acid content in the roots of *T. chinensis* var. *mairei* seedlings.

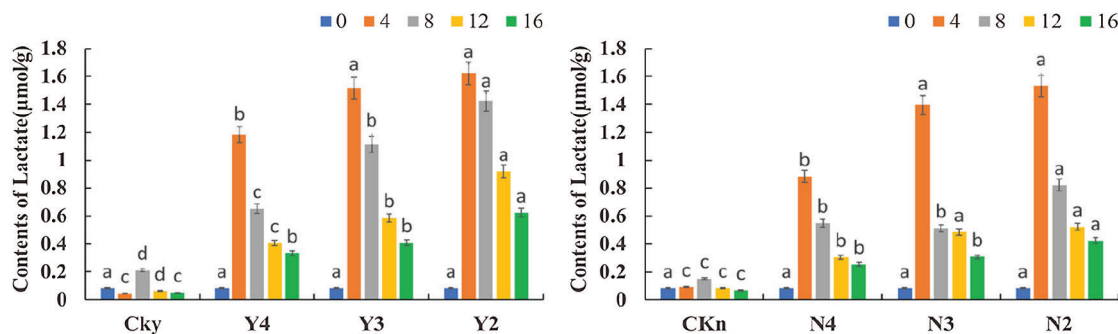


Figure 10: Lactic acid content in inoculated (CKy, Y4, Y3, and Y2) and uninoculated (CKn, N4, N3, and N2) roots of *T. chinensis* var. *mairei* under water stress after 4, 8, 12, and 16 days of watering

Note: Differences between the treatment groups in the figure were analyzed with the same number of days. Different letters from the same group indicate significant differences ($P < 0.05$)

4 Discussion

4.1 *P. indica* Colonization in the Roots of *T. chinensis* var. *mairei* and Comparison of Plant Growth under Different Degrees of Water Stress

The *T. chinensis* var. *mairei* seedlings inoculated with *P. indica* did not have chlamydo spores on the 15th day after inoculation. After 40 d, more chlamydo spores were observed in the roots, and the root hairs and the root system substantially increased. This result was consistent with that of Liang et al. [27], who reported that inoculation of *P. indica* on *Medicago truncatula* notably promotes root growth at 15–30 d. It also showed that gymnosperms have a longer infection of the test mycorrhiza after inoculation with *P. indica*.

As water stress intensified, the growth of the *T. chinensis* var. *mairei* seedlings and their roots gradually deteriorated, indicating that this species is not tolerant to flooding and water stress is not conducive to its growth. This finding was consistent with that of Ivanov et al. [28], who reported that pine and spruce seedlings grow poorly and even die under water stress.

4.2 Effects of *P. indica* on Root Vitality and Respiratory Substrates in the Roots of *T. chinensis* var. *mairei* under Water Stress

Under different water stress treatments, the *T. chinensis* var. *mairei* seedlings had substantially decreased root vitality. The inoculated *T. chinensis* var. *mairei* seedlings had higher root vitality and slower reduction rate in this index than the uninoculated seedlings, indicating that *P. indica* can increase the root vitality of *T. chinensis* var. *mairei* and reduce the damage to its roots caused by water stress. This result was consistent with that of Ghodrat et al. [29], who noted that the root vitality of cotton declines under water stress.

The *T. chinensis* var. *mairei* seedlings inoculated with *P. indica* had a higher soluble sugar content in the roots than the uninoculated seedlings. Moreover, its soluble sugar content increased as water stress intensified. This observation was consistent with that of Khosravi et al. [30], who found that the soluble sugar content of wheat increases under flooding stress. Soluble sugars can adjust the osmotic pressure in plants, indicating that inoculation with *P. indica* helps retain the photosynthetic metabolites of seedlings, thereby increasing the osmotic adjustment capacity of the root system.

4.3 Effects of *P. indica* on the Activity of Aerobic Respiratory Metabolic Enzymes and Products in the Roots of *T. chinensis* var. *mairei* under Water Stress

The *T. chinensis* var. *mairei* seedlings inoculated with *P. indica* had a higher MDH and SDH activity and higher malic acid content in the roots than the uninoculated seedlings, indicating that *P. indica* can increase the aerobic respiration rate of *T. chinensis* var. *mairei*. As water stress further intensified over time, the MDH and SDH activity and the content of malic acid of the *T. chinensis* var. *mairei* seedlings gradually decreased, suggesting that the aerobic respiration in the roots of this species gradually decreased under water stress. This finding was consistent with that of Guibin et al. [31], who reported that the MDH activity and aerobic respiration intensity in the roots of *Eucalyptus* decreases under flooding stress, as well as with that of Pereira et al. [32], who observed that the malic acid content in the roots of Spinach gradually decreases under flooding stress.

4.4 Effects of *P. indica* on the Activity of Anaerobic Respiratory Metabolic Enzymes and Products in the Roots of *T. chinensis* var. *mairei* under Water Stress

Under water stress, the ADH activity and the lactic acid content in the roots initially substantially increased and then decreased. Moreover, LDH activity increased as was stress intensified. This result was similar to that of Du et al. [33], who reported that the ADH activity of wheat seedling roots initially increases and then stabilizes under waterlogging stress. These findings indicated that *T. chinensis* var. *mairei* has a limited aerobic respiration under water stress and instead mainly performs anaerobic respiration. In addition, the inoculated *T. chinensis* var. *mairei* seedlings had a higher LDH and ADH activity and a higher lactic acid content in the roots than the uninoculated seedlings. This trend was similar to that observed by Li et al. [34], who found that white clover has a high lactic acid content in the roots under hypoxia, indicating that *P. indica* plays a positive role in enhancing the antihypoxic ability of *T. chinensis* var. *mairei*.

To sum up, this study demonstrated that *P. indica* can enhance the root vitality and waterlogging resistance of *T. chinensis* var. *mairei*. This work provides a theoretical basis and scientific method for promoting and planting *T. chinensis* var. *mairei* in humid areas in southern China. However, this study has several limitations. Only *T. chinensis* var. *mairei* was selected in studying respiration under water stress. Follow-up studies should include other species of *Taxus* or other precious tree species. Moreover, the mechanism of the effect of *P. indica* on the respiration of *T. chinensis* var. *mairei* under water stress must be investigated further. Given that the regulation of respiratory metabolism under hypoxic environment involves a more complicated mechanism, future studies should focus on the molecular level and resolve related problems via biochemical or molecular technologies.

Funding Statement: This work was supported by the National Natural Science Foundation of China (No. 31270740).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Gao, R. M., Shi, X. D., Fan, L. Y., Sun, Y. Y., Guo, X. H. (2016). Natural distribution and community ecological characteristics of *taxus chinensis* var. *mairei* in Shanxi Province, China. *The Journal of Applied Ecology*, 27(6), 1820. DOI 10.13287/j.1001-9332.201606.029.
2. Zhang, D. Q., Zhou, N. (2013). Genetic diversity and population structure of the endangered conifer *taxus wallichiana* var. *mairei* (Taxaceae) revealed by simple sequence repeat (SSR) markers. *Biochemical Systematics and Ecology*, 49, 107–114. DOI 10.1016/j.bse.2013.03.030.

3. Qiao, W., Ling, F., Yu, L., Huang, Y., Wang, T. (2017). Enhancing taxol production in a novel endophytic fungus. *Aspergillus Aculeatinus Tax-6, Isolated from Taxus Chinensis var. Mairei*. *Fungal Biology*, 121(12), 1037–1044. DOI 10.1016/j.funbio.2017.08.011.
4. Nimasow, G., Nimasow, O. D., Rawat, J. S., Tsering, G., Litin, T. (2016). Remote sensing and GIS-based suitability modeling of medicinal plant (*Taxus baccata* linn.) in tawang district, arunachal pradesh, India. *Current Science*, 110(2), 219–227. DOI 10.18520/cs/v110/i2/219-227.
5. Wei, Q., Yin, C. W. (2019). Chemical composition of essential oils from the stems of *taxus chinensis* var. *mairei*. *Journal of Essential Oil Bearing Plants*, 22(4), 1144–1149. DOI 10.1080/0972060X.2019.1668864.
6. Song, L. L., Zhang, H. N., Zhao, H. Q., Jiang, Y. L., Hou, M. F. (2014). In vitro germination and seedling development of *taxus chinensis* var. *mairei* by embryo culture. *Journal of Agricultural Science & Technology*, 16, 1355–1363. <https://www.researchgate.net/publication/288536196>.
7. Jian, Z. Y., Meng, L., Wang, N., Xu, G. F., Shi, Y. (2016). Characteristic and protection of rare and endangered *taxus chinensis* var. *mairei* in the taihang mountains. *Nutrición Hospitalaria*, 33(3), 698–702. <https://www.redalyc.org/articulo.oa?id=309246400029>.
8. Lin, H. F., Xiong, J., Zhou, H. M., Chen, C. M., Lin, F. Z. et al. (2019). Growth promotion and disease resistance induced in *anthurium* colonized by the beneficial root endophyte *piriformospora indica*. *BMC Plant Biology*, 19(1), 40. DOI 10.1186/s12870-019-1649-6.
9. Gosal, S. K., Sharma, M., Gosal, S. S., Chhibba, I. M., Bhatnagar, K., Varma, A. (2011). Biohardening with *piriformospora indica* improves survival rate, growth, iron uptake and cane yield of micropropagated sugarcane. *International Sugar Journal*, 113(1349), 382–388. <https://www.redalyc.org/articulo.oa?id=309246400029>.
10. Li, Q., Kuo, Y. W., Lin, K. H., Huang, W., Deng, C. et al. (2021). Piriformospora indica colonization increases the growth, development, and herbivory resistance of sweet potato (*Ipomoea batatas* L.). *Plant Cell Reports*, 40(2), 339–350. DOI 10.1007/s00299-020-02636-7.
11. Das, A., Kamal, S., Shakil, N. A., Sherameti, I., Oelmüller, R. et al. (2012). The root endophyte fungus *piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *coleus forskohlii*. *Plant Signaling & Behavior*, 7(1), 103–112. DOI 10.4161/psb.7.1.18472.
12. Kumar, M., Yadav, V., Kumar, H., Sharma, R., Singh, A. et al. (2011). *Piriformospora indica* enhances plant growth by transferring phosphate. *Plant Signaling & Behavior*, 6(5), 723–725. DOI 10.4161/psb.6.5.15106.
13. Candan, N., Tarhan, L. (2012). Tolerance or sensitivity responses of *mentha pulegium* to osmotic and waterlogging stress in terms of antioxidant defense systems and membrane lipid peroxidation. *Environmental and Experimental Botany*, 75, 83–88. DOI 10.1016/j.envexpbot.2011.08.014.
14. Knapp, A. K., Hoover, D. L., Wilcox, K. R., Avolio, M. L., Koerner, S. E. et al. (2015). Characterizing differences in precipitation regimes of extreme wet and dry years: Implications for climate change experiments. *Global Change Biology*, 21(7), 2624–2633. DOI 10.1111/gcb.12888.
15. Knapp, A. K., Avolio, M. L., Beier, C., Carroll, C. J., Collins, S. L. et al. (2017). Pushing precipitation to the extremes in distributed experiments: Recommendations for simulating wet and dry years. *Global Change Biology*, 23(5), 1774–1782. DOI 10.1111/gcb.13504.
16. Bárzana, G., Carvajal, M. (2020). Genetic regulation of water and nutrient transport in water stress tolerance in roots. *Journal of Biotechnology*, 324, 134–142. DOI 10.1016/j.jbiotec.2020.10.003.
17. Niu, X., Hu, T., Liu, T., Wu, X., Feng, P. et al. (2014). Appropriate partial water stress improving maize root absorbing capacity. *Transactions of the Chinese Society of Agricultural Engineering*, 30(22), 80–86. DOI 10.3969/j.issn.1002-6819.2014.22.010.
18. Zhang, Y. J., Xie, Z. K., Wang, Y. J., Su, P. X., An, L. P. et al. (2011). Effect of water stress on leaf photosynthesis, chlorophyll content, and growth of oriental lily. *Russian Journal of Plant Physiology*, 58(5), 844. DOI 10.1134/S1021443711050268.
19. Ekmekci, Y., Bohms, A., Thomson, J. A., Mundree, S. G. (2005). Photochemical and antioxidant responses in the leaves of *xerophyta viscosa* baker and *digitaria sanguinalis* L. under water deficit. *Zeitschrift für Naturforschung*, 60(5–6), 435–443. DOI 10.1515/znc-2005-5-612.

20. Seo, C., Lee, S., Kang, S., Park, Y., Kim, A. et al. (2017). Selection of suitable plant growth regulators for augmenting resistance to waterlogging stress in soybean plants (*Glycine max* L.). *Korean Journal of Crop Science*, 62(4), 325–332. <https://www.dbpia.co.kr/journal/articleDetail?nodeId=NODE07470438>.
21. Pereira, A. (2016). Plant abiotic stress challenges from the changing environment. *Frontiers in Plant Science*, 7, 1123. DOI 10.3389/fpls.2016.01123.
22. Cao, J., Liu, C., Wu, Y., Li, H., Li, M. (2017). Geographic distribution and ecological zoning of *taxus chinensis* var. *mairei* in China. *Acta Horticulturae*, 1185, 265–276. DOI 10.17660/ActaHortic.2017.1185.34.
23. Hawrylak-Nowak, B., Matraszek, R., Pogorzelec, M. (2015). The dual effects of two inorganic selenium forms on the growth, selected physiological parameters and macronutrients accumulation in cucumber plants. *Acta Physiologiae Plantarum*, 37(2), 41. DOI 10.1007/s11738-015-1788-9.
24. Wei, F., Zheng, Q. K., Luo, S. Q., Qiu, J., Yang, W. F. (2014). A method for measuring soluble sugars and starch in bark and xylem of rubber tree. *Chinese Journal of Tropical Agriculture*, 4, 12–16. DOI CNKI: SUN: RDNK.0.2014-04-002.
25. Ma, B., Chen, J., Zheng, H., Fang, T., Ogutu, C. et al. (2015). Comparative assessment of sugar and malic acid composition in cultivated and wild apples. *Food Chemistry*, 172, 86–91. DOI 10.1016/j.foodchem.2014.09.032.
26. Sales, C. R., da Silva, A. B., Carmo-Silva, E. (2020). Measuring rubisco activity: Challenges and opportunities of NADH-linked microtiter plate-based and ¹⁴C-based assays. *Journal of Experimental Botany*, 71(18), 5302–5312. DOI 10.1093/jxb/eraa289.
27. Li, L., Li, L., Wang, X., Zhu, P., Wu, H. et al. (2017). Plant growth-promoting endophyte *piriformospora indica* alleviates salinity stress in *medicago truncatula*. *Plant Physiology and Biochemistry*, 119, 211–223. DOI 10.1016/j.plaphy.2017.08.029.
28. Ivanov, Y., Kartashov, V., Zlobin, E., Kuznetsov, V. (2018). Water deficit-dependent changes in non-structural carbohydrate profiles, growth and mortality of pine and spruce seedlings in hydroculture. *Environmental and Experimental Botany*, 157, 151–160. DOI 10.1016/j.envexpbot.2018.10.016.
29. Hamidi, R., Sharafzadeh, S., Ghodrati, V., Bazrafshan, F., Alizadeh, O. (2016). Effect of water stress at flowering stage on morphophysiological characteristics of cotton (*Gossypium hirsutum* L.) cultivars in southern Iran. *ecology. Environment and Conservation*, 22, 1763–1773. <https://www.researchgate.net/publication/317204820>.
30. Khosravi, M. S., Heidari, R., Jamei, R., Kouhi, S. M. M., Moudi, M. (2018). Comparative growth and physiological responses of tetraploid and hexaploid species of wheat to flooding stress. *Acta Agriculturae Slovenica*, 111(2), 285–292. DOI 10.14720/aas.2018.111.2.04.
31. de Sá Martins, R., Faria, J. M. R., Rossini, B. C., Marino, C. L., Dos Santos, L. D. et al. (2020). Proteomic analyses unraveling water stress response in two *eucalyptus* species originating from contrasting environments for aridity. *Molecular Biology Reports*, 47(7), 5191–5205. DOI 10.1007/s11033-020-05594-1.
32. Pereira, C., Dias, M. I., Petropoulos, S. A., Plexida, S., Chrysargyris, A. et al. (2019). The effects of biostimulants, biofertilizers and water-stress on nutritional value and chemical composition of Two spinach genotypes (*Spinacia oleracea* L.). *Molecules*, 24(24), 4494. DOI 10.3390/molecules24244494.
33. Du, H. Y., Liu, D. X., Liu, G. T., Liu, H. P., Kurtenbach, R. (2018). Relationship between polyamines and anaerobic respiration of wheat seedling root under water-logging stress. *Russian Journal of Plant Physiology*, 65(6), 874–881. DOI 10.1134/S1021443718060055.
34. Li, Z., Cheng, B., Yong, B., Liu, T., Peng, Y. et al. (2019). Metabolomics and physiological analyses reveal β-sitosterol as an important plant growth regulator inducing tolerance to water stress in white clover. *Planta*, 250(6), 2033–2046. DOI 10.1007/s00425-019-03277-1.