SYMBIOSIS (2007) 43, 21–28 ©2007 Balaban, Philadelphia/Rehovot ISSN 0334-5114

Extensive host range of an endophytic fungus affects the growth and physiological functions in rice (*Oryza sativa* L.)

Zhi-Lin Yuan[1,3], Chuan-Chao Dai[1*], Xia Li[2], Lin-Shuang Tian[1], and Xing-Xiang Wang[4]

[1]Jiangsu Key Laboratory for Biological Diversity and Biotechnology, College of Life Science, Nanjing Normal University, Nanjing 210097, Jiangsu Province, China, Emails. zhi_lin_yuan@163.com and daichuanchao@njnu.edu.cn;
[2]Institute of Agrobiological Genetics and Physiology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu Province, China;
[3]Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang 311400, Zhejiang Province, China;
[4]Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, Jiangsu Province, China

(Received November 15, 2006; Accepted January 29, 2007)

Abstract
Endophytic *Phomopsis* sp. was isolated from the inner bark of *Bischofia polycarpa*. Many previous studies indicated that the endophyte was ubiquitous and located within extensive host ranges, and indirect evidence had shown that *Phomopsis* sp. might be an "inducible mutualism" endophyte and horizontally transmitted. Nevertheless, little attention was paid to whether an inducible endophyte can locate into non-host plant tissues, and how it influences plant physiological mechanisms still remains unclear. In the present study, we attempted to inoculate the endophyte into rice, hypothesizing that inducible endophytes could have particular beneficial effects on non-host plants. Inoculation and infection tests proved that the endophyte established a symbiotic relationship with the rice plant and existed in the leaf tissue. Then we tested the hypothesis in two ways. First, the effects of diluted endophyte inoculum on the germination of seeds were evaluated. Second, at the seedling stage (4-leaf stage), seedlings in pots were treated with the endophyte inoculum under controlled environmental conditions. Parameters including numbers of tillers, plant height, chlorophyll content, photosynthetic rate, grain yield, and antioxidant enzyme activity were measured. These parameters differed between endophyte-infected and endophyte-free plants, especially at the germination and seedling stages, being significantly greater in endophyte-infected plants; however, the endophyte made no obvious contribution to the grain yield of rice. The results revealed that the endophyte promoted growth, antioxidant enzyme activity, and photosynthesis. Therefore, endophytic *Phomopsis* sp. may be useful as a growth-promoting microbial agent for enhancing the vigor and optimizing the quality of plants in agriculture, horticulture, and forestry.

Keywords: Extensive host range, endophytic fungi, rice, symbiosis

1. Introduction

Endophytic fungi, defined as fungi living inside healthy plant tissues, are now considered to be ubiquitous symbionts of plants that do not produce disease symptoms. Nowadays, endophytic fungi are of biotechnological interest due to their potential use as genetic vectors (Murray et al., 1992), as biological control agents (Dorworth and Callan, 1996; Arnold et al., 2003) and as sources of secondary metabolites (Stierle et al., 1993; Strobel et al., 2003).

Most studies focus on the relationships and interactions between grass endophytes and their host plants, such as two economic grass crops, *Festuca arundinacea* Shreb. and *Lolium pernne* Li. (White et al., 1985; Spyreas et al., 2001), which are the model plants for study of plant-endophyte associations. These associations may have negative, neutral, or positive consequences. Some grass endophytes (systemic endophytes) mainly follow a vertical transmission model via seeds. On the other hand, tree endophytes (inducible endophytes) are horizontally transmitted via fungal spores from plant to plant (Saikkonen et al., 2004; Carroll et al., 1988). Little attention has been paid to the horizontal transmission model of some fungal endophytes relative to their vast diversity, and whether they can locate into non-host plant tissues and influence the plant's physiological mechanisms in a similar way remains unclear.

Here, we describe an endophytic fungus that was isolated from the inner bark of *Bischofia polycarpa* (not found in seeds) and identified as *Phomopsis* sp. Our

---

*The author to whom correspondence should be sent.

previous works have shown that the endophytic fungus could secrete abundant lassase and cellulase (Shi et al., 2004). Endophytic fungi synthesize a series of enzymes necessary for penetrating and colonizing their plant hosts (Petrini et al., 1992); lassase and cellulase are assumed to be responsible for the degradation of cellulose and lignin in the plant cell wall. Many studies have also verified that the host ranges of the genus *Phomopsis* sp. were extensive (Murali et al., 2006; Wadia et al., 2000; Zou et al., 2001; Dai et al., 2005). We also reported that the weight of *Euphorbia pekinensis* roots and seedlings increased after they were inoculated with endophytic *Phomopsis* sp. (Dai et al., 2005). Further experiments examined the 3-indoleacetic acid (IAA), abscisic acid (ABA), and VB1 produced by the endophyte *in vitro* (Yuan et al., 2004). *Phomopsis* sp. may be a latent plant pathogen that causes many diseases in agriculture. On the other hand, *Fusarium equiseti*, *F. oxysporum,* and *Phoma sorghina* were also reported as latent endophytic pathogens in rice without causing disease symptoms previously (Fisher et al., 1992). The purpose of this study is to address whether *Phomopsis* sp. is a mutualistic, antagonistic, or commensalistic endophyte in rice and determine the morphological and physiological response of the rice plant to the endophytic fungus inoculation. This information can be used to guide cultivation and develop new uses of the endophytic fungus to improve the physiological functions of extensive plant tissues.

2. Materials and Methods

Fungal strain and rice seed

The rice seeds came from Jiangsu Academy of Agricultural Sciences. The endophytic *Phomopsis* sp. is conserved in the Jiangsu Key Laboratory for Biological Diversity and Biotechnology, College of Life Science, Nanjing Normal University.

Seed germination

The endophytic *Phomopsis* sp. was cultured in broth at 25°C, stirring the culture at 150 r/min for 3 days, and diluted before inoculation. The liquid fermentation medium contained 200 g potato extract, 20 g sodium carboxymethyl cellulose (CMC-Na), 1 g NH_4Cl, 3 g KH_2PO_4, 1.5 g $MgSO_4 \cdot 7H_2O$, and 7 ml mixed solution of trace metals (per liter). The trace element composition was as follows: aminoacetic acid 7.8×10^{-3} mol/l, $MnSO_4 \cdot H_2O$ 2.9×10^{-3} mol/l, NaCl 1.7×10^{-2} mol/l, $FeSO_4 \cdot 7H_2O$ 3.59×10^{-4} mol/l, $CoCl_2$ 7.75×10^{-4} mol/l, $CaCl_2 \cdot 2H_2O$ 9.0×10^{-4} mol/l, $ZnSO_4 \cdot 7H_2O$ 3.48×10^{-4} mol/l, $KAl(SO_4)_2 \cdot 12H_2O$ 2.1×10^{-5} mol/l, HBO_3 1.6×10^{-4} mol/l, $NaMnO_4$ 4.1×10^{-5} mol/l. The fungal liquid culture (8.41 g DW/l) was diluted 2-fold, 3-fold, and 4-fold, respectively. Rice seeds were soaked in water for 2 days at 27°C, then germinated on a Petri dish (diameter = 11 cm, 50 grains per culture dish). When the seeds sprouted, 20 ml mycelium liquid culture was added to each dish, except the control group, which used tap water. In total, 4 treatments with 3 replicates were designed. The seminal root length, adventitious root number, seedling height, and seedling fresh weight were determined after incubation at 27°C for 6 days.

Ex vitro *plant inoculation and re-isolation of fungal endophyte*

The seedlings were planted in pots filled with autoclaved sterilized soil (121°C for 20 min), and 50 ml mycelium liquid culture (as above) was added to the soil around each plant. Leaves, sheaths, and roots were collected 7 days later. Samples were rinsed with 75 % ethanol, then disinfected in 0.1% $HgCl_2$ for 5 minutes. We transferred these samples to the PDA media for culture at 27°C to isolate endophytic fungi. The mycelium of the endophytic fungus in the leaf tissue was observed with a staining technique (Bacon et al., 1977).

Pot experiment design

The rice seeds were sterilized in 5% H_2O_2 for 5 minutes, soaked in water for 24 h, incubated at 35°C for 48 h, and finally sowed by stage. Seedlings at similar developmental stages were transplanted into pots (5 hills per pot, 1 seedling per hill) and grown in an outdoor net room at the Jiangsu Academy of Agriculture Sciences. A completely randomized design with five replicates was utilized. Average temperature varied from 21°C to 27°C, with daily temperature differences from 7.1°C to 8.7°C. Chemical fertilizer was applied with a combination of 2.0 g N, 1.6 g P_2O_5, and 1.4 g K_2O per plot as basal dressing and 1.0 g N as top dressing at the tillering and booting stages. The soil type was paddy soil. At the seedling stage (4-leaf stage), each seedling was treated with 100 ml endophytic mycelium culture (original fungal concentration). Starting 20 days later, the numbers of tillers and height change of seedlings were evaluated at 10-day intervals (25 seedlings measured from E+ and E–, respectively). Plants were watered and fertilized regularly during the growing season (from May 1 to October 15). Grain yield was measured after harvesting all plants in pots.

Enzyme assays of leaves

Leaf tissue (0.5 g) was harvested from each plant and quickly homogenized with 5 ml cold 50 mmol/l phosphate (pH 7.8) extraction buffer using a mortar and pestle that were pre-chilled in an ice bath. The slurry was centrifuged at 15,000 *g* for 20 min at 4°C and the supernatants were used for enzyme assays. Total SOD (superoxide dismutase: EC 1.15.1.1) activity was assayed by the method described in Giannopolitis and Ries (1977). Total POD (peroxidase: EC 1.11.1.7) activity was assayed as described previously in Zhang (1990).

Lipid peroxidation measurement

Leaves (0.5 g) were homogenized in 4 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 g for 20 min. Lipid peroxidation was measured as MDA (malondialdehyde) determined by the thiobarbituric acid (TBA) reaction according to the method described in Madhava and Sresty (2000).

Measurement of chlorophyll content

A leaf disc was immersed in 80% acetone and kept in the dark until the disc completely faded. The chlorophyll content of the disc was determined with a UV-754 ultraviolet spectrophotometer according to the method of Wellburn and Lichtenthaler (1984).

Measurement of net photosynthetic rate

Net photosynthetic rate (*Pn*) was measured when flag leaves were fully expanded using an open gas exchange system (LI-6400, LI-COR Inc., USA) (Peng et al., 2002). Measurements were made at the middle region of intact flag leaf blades and an air CO_2 concentration of 350 μmol mol^{-1} was maintained during measurement. Three leaves of the rice plants selected from each replicate were analyzed for net photosynthetic rate.

Statistical analysis

Data were statistically processed by analysis of variance (ANOVA) using the SPSS package according to the method of Mucciarelli (2003). Differences were considered significant when $P<0.05$ or $P<0.01$ (probability level). SD (Standard Deviation) was calculated and shown in the figures and tables.

3. Results

Re-isolation and distribution of endophytic fungus in rice seedlings

As shown in Fig. 1, a fungus colony appeared from leaf tissue on PDA after incubation for 4 days at 27°C. In order to test whether the colony was identical to the endophytic fungus, we transferred the colony to fresh PDA medium. We found that mycelium morphology and rear color of the colony were identical to the original endophytic *Phomopsis* sp. Figs. 1B and 1C represent the distribution of the endophytic *Phomopsis* sp. in the leaf tissue. Hyphae were not detected in the roots or sheath, indicating that the endophyte colonization was tissue-specialized. Meanwhile, endophyte-colonized tissues remained asymptomatic, proving the establishment of inducible endophytic fungus as a symbiont of rice seedlings.

Table 1.
Effects of different concentrations of endophyte inoculum on the germination of rice seeds.

	Seminal root length (cm)	Lateral root number (n)	Adventitious root number (n)	Seedling height (cm)	Seedling fresh weight (mg)	Germination percentage (%)
Control	2.74 ± 0.71^a	13.96 ± 2.01^a	5.40 ± 0.61^a	2.53 ± 0.04^a	56.7 ± 4.2^a	84.67 ± 1.53^a
2-fold diluted	5.36 ± 0.83^b	24.08 ± 8.05^a	5.25 ± 0.13^a	3.37 ± 0.48^b	65.2 ± 3.5^b	76.00 ± 7.00^a
3-fold diluted	5.10 ± 1.45^b	27.57 ± 6.79^b	4.56 ± 0.57^a	3.28 ± 0.30^b	68.8 ± 3.9^b	79.67 ± 4.73^a
4-fold diluted	5.60 ± 0.71^b	27.79 ± 5.02^b	5.09 ± 0.89^a	3.16 ± 0.11^b	68.6 ± 2.2^b	77.67 ± 2.08^b

Values are means ± SD with three replicates. Means followed by the same letter in a column within each variety are not significantly different by LSD at P=0.05.

Table 2. Comparison of grain yield and related parameters between E+ and E– population.

	E+	E–	% of control
Plant height (cm)	95.64 ± 4.63^a	94.56 ± 4.27^a	101
Panicles per plant (n)	9.17 ± 4.92^a	6.83 ± 2.64^a	142
Panicle length (cm)	18.24 ± 1.24^a	17.26 ± 1.26^a	105
Filled grains per panicle (n)	105.49 ± 15.53^a	105.19 ± 16.86^a	100
Seed set (%)	87.96 ± 4.72^a	85.72 ± 7.20^a	103
1000-seed weight (g)	27.14 ± 1.58^a	26.55 ± 0.87^a	102
Grain yield (g. $plant^{-1}$)	23.21 ± 9.83^a	19.71 ± 8.45^a	118
Above ground dry weight (g)	55.24 ± 21.21^a	51.72 ± 11.9^a	107

Values are means ± SD with five replicates.

Effects of different diluted endophyte inoculum on the germination of rice seeds

The data represented in Table 1 show that the seeds grew vigorously after the addition of endophyte inoculum. The seminal root length, lateral root number, height, and fresh weight of seedlings all increased significantly compared to the control group. The germination percentage of seeds treated with endophyte was a little lower than the control; this could be due to the fact that the seed surfaces were covered with endophytic inoculum and respiration of seeds was restrained to a certain extent.

The numbers of tillers and plant growth rate of rice seedlings

Fig. 2 shows that numbers of tillers and seedling growth rate were equal in endophyte-infected and noninfected seedlings at day 20 after inoculation. However, at days 30 and 40 after inoculation, the number of tillers and growth rate were significantly ($P<0.05$) larger for endophyte-infected seedlings than for noninfected seedlings.

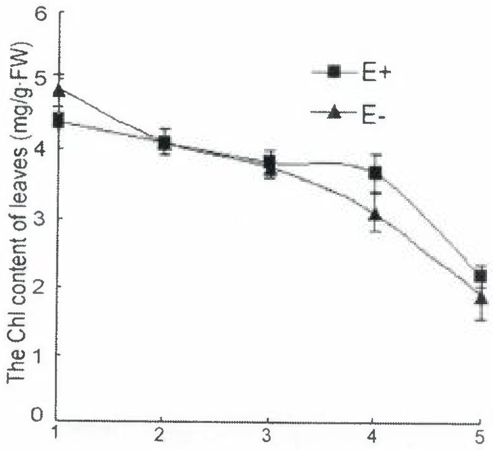

Figure 3. Comparison of the Chl content dynamics of leaves between E+ and E– populations. 1: tillering stage, 2: booting stage, 3: heading stage, 4: grain-filling stage, 5: ripening stage.

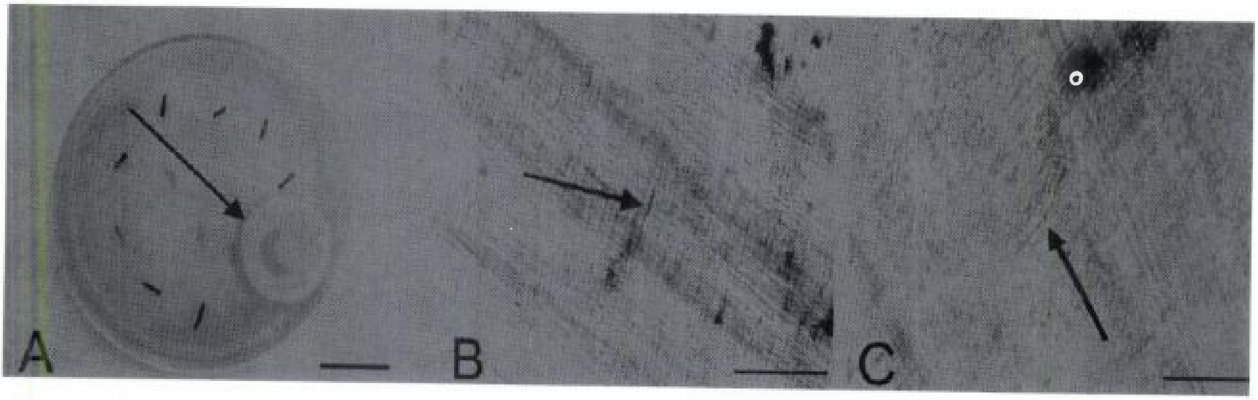

Figure 1. The endophytic *Phomopsis* sp. was isolated from the leaf tissue and the mycelium was observed with staining technique. (A: Fungal colony, Bar=2.5 cm; B and C: Distribution of endophytic mycelium in leaf tissue, Bars=50 μm and 25 μm).

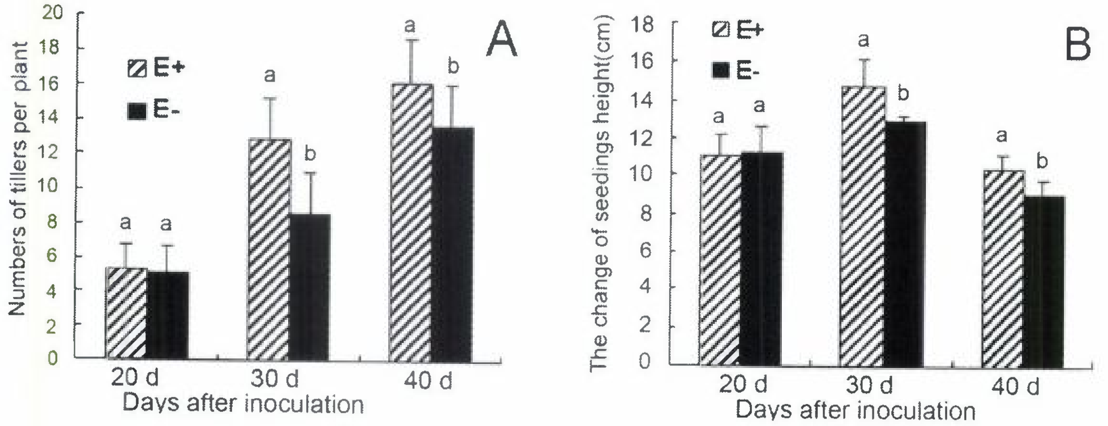

Figure 2. Comparison of the numbers of tillers (A) and the change in seedling height (B) between E+ and E– populations. Values are means of 25 samples. Mean values in each frame followed by different letters are significantly different at $P<0.05$.

Effects of endophytic fungus on the grain yield of rice

With respect to the rice production and related parameters, no remarkable differences in grain yield, panicle length and other parameters were registered between E+ and E– populations (Table 2).

The chlorophyll content and net photosynthetic rate of leaves

With respect to changes in Chl level (Fig. 3), it showed a declining trend over the whole growth stage. The Chl content of leaves inoculated with endophyte was a little lower than that of noninfected plants in the tillering stage, but surpassed it in the grain-filling stage and equaled it at the last sampling dates. However, there was no significant difference between the two populations.

Fig. 4 shows the different net photosynthetic rates of leaves in endophyte-infected and non-infected plants. Especially at 9:30 am and 12:30 pm (under PFD of 1500 μm^{-2} s^{-1} and 1160 μm^{-2} s^{-1}, respectively), *Pn* was significantly higher in infected than in noninfected plants, and the difference reached the significant level (at P=0.05 and P=0.01). However, there was no obvious variance in *Pn* between endophyte-infected and noninfected plants at other times. The relative *Pn* increase in the endophyte-infected plants was 10.6% throughout an entire day. We speculate that endophyte-infected plants may adaptively respond to increasing temperature and high light intensity.

Analysis of SOD and POD enzyme activity and MDA content of leaves between E+ and E– seedlings

At the seedling stage, SOD and POD enzyme activity in

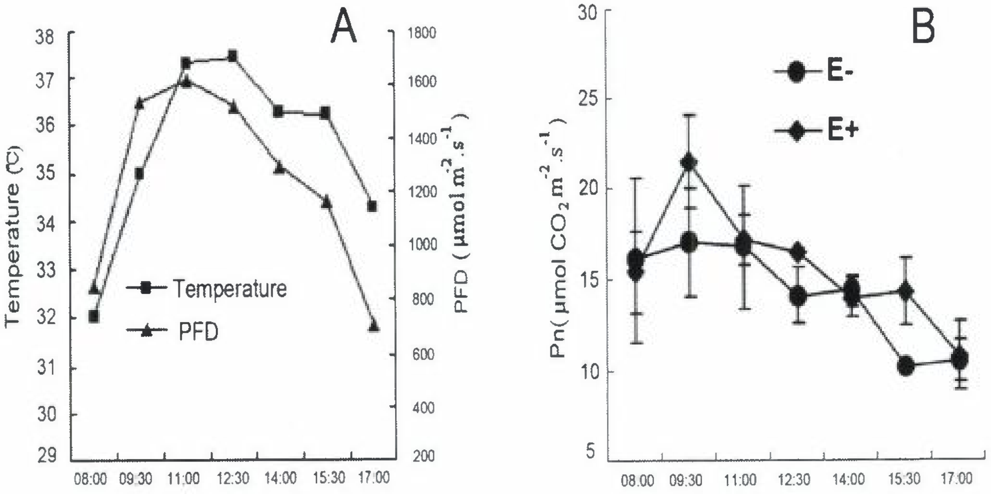

Figure 4. Diurnal changes in temperature and photo flux density (PFD) are demonstrated (A). Diurnal changes in photosynthetic rate of leaves with E+ and E– populations at grain-filling stage (B).

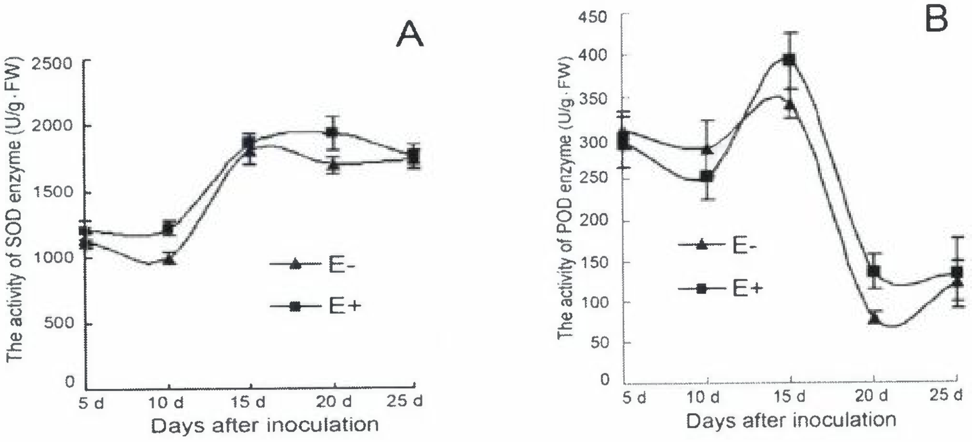

Figure 5. Activity of SOD and POD enzymes in leaves at the seedling stage after inoculation with endophytic fungus. Values are the mean ± SD of five replicates.

leaves were efficiently stimulated by the endophyte and reached peak values after inoculation for 20 days and 15 days respectively. This was significantly different from noninfected seedlings ($P<0.05$). Twenty days after inoculation, the SOD enzyme activity of endophyte-treated seedlings was remarkably higher than that of non-inoculated seedlings ($P<0.01$). From the curves in Fig. 6, we could conclude that SOD and POD enzyme activity was induced by the endophytic fungus at all developmental stages and MDA content was maintained at a comparatively low level. Compared to the E– populations, SOD and POD enzyme activity in E+ populations increased by 9.4% and 19.7%, while MDA content was reduced by 12.7%. However, there was no significant variance within the two populations.

4. Discussion

To our knowledge, this paper is the first report of an established mutualistic association between rice and inducible endophytic fungus. Extensive previous research has been done on the physiological interactions between plants and their own endophytes, but very little is known about how inducible (non-systemic) endophytes affect other plants. More detailed knowledge of whether an endophyte can exist in non-host plants would help us understand its physiological and ecological roles. Therefore, to address the question of whether *Phomopsis* sp. could exist as a mutualistic fungus in rice, we inoculated endophytic *Phomopsis* sp. into rice seedlings and investigated how this fungus altered the physiological, developmental, and morphological properties of the rice. Endophytic fungi provide their host plants with many competitive advantages over endophyte-free plants: for instance, they can promote growth, regulate the plant's protective enzyme system, and give resistance to disease attack (Mucciarelli et al., 2003; Kranner et al., 2005; Yates et al., 1997; Waller et al., 2005; Yuan et al., 2005). *Piriformospora indica*, a new root endophyte, was recently found to have growth-promoting effects on a vast range of plant hosts (Varma et al., 2001). Our results are consistent with the above conclusions and show that *Phomopsis* sp.'s interaction with plants is similar to that of *Piriformospora indica*.

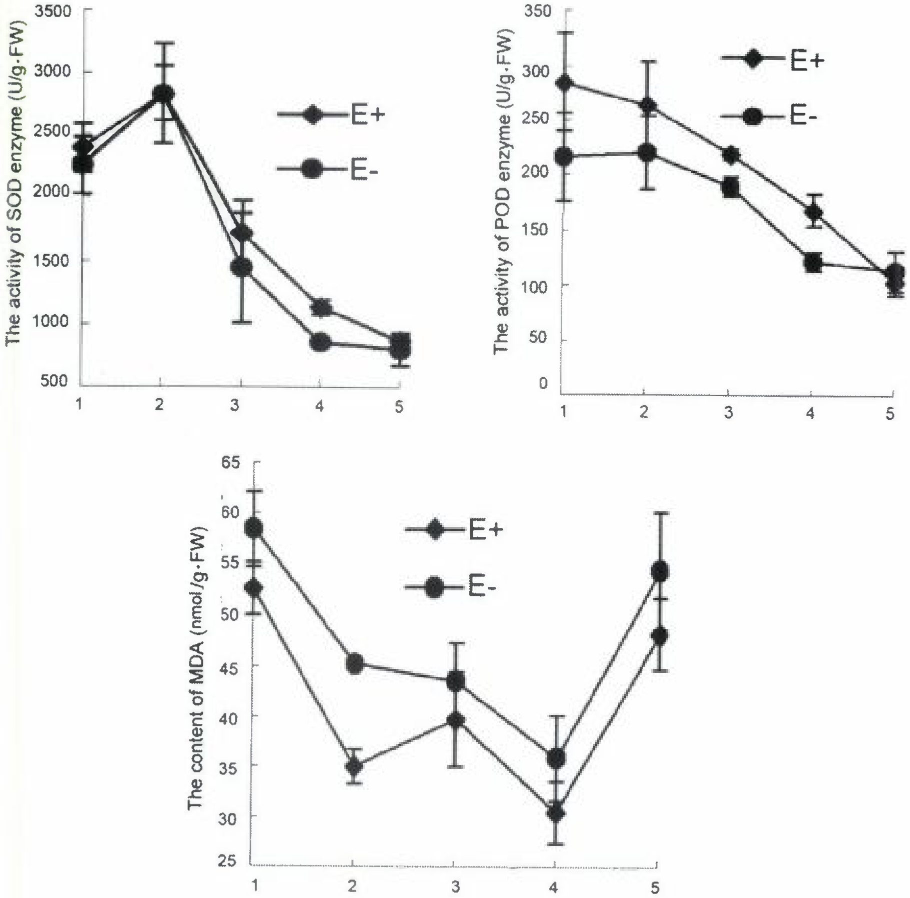

Figure 6. SOD and POD enzyme levels and MDA content of leaves during the whole growth stage. Values are the mean ± SD of five replicates. 1: tillering stage, 2: booting stage, 3: heading stage, 4: grain-filling stage, 5: ripening stage.

Most of the parameters we measured differed between endophyte-infected and endophyte-free plants, especially at the germination and seedling stages; the endophytic *Phomopsis* sp. promoted growth and notably increased the number of tillers of seedlings.

Antioxidation SOD and POD enzymes are active oxygen scavengers that alleviate the damage done by active oxygen to lipids. The endophytic *Phomopsis* sp. efficiently increased SOD and POD enzyme activity in rice, especially at the seedling stage. The accumulation of MDA in infected plants also decreased. Therefore, we hypothesized that inoculation with endophytic fungus might be an effective way to further increase the photosynthetic rate and reduce photooxidation under environmental stress.

Few researchers have referred to the effects of endophytic fungi on photosynthesis in the host, but controversy exists on this topic (Belesky et al., 1987; Smith et al., 1985; Pinto et al., 2000; Thrower et al., 1973). Photosynthetic rate is affected by many environmental factors, as well as by the host genotype. In our study, endophytic *Phomopsis* sp. increased the photosynthetic rate of rice at specific times during the grain-yield stage, possibly due to the relatively high level of antioxidant enzymes preventing photo-damage under high irradiance and temperature (Logan et al., 2006). However, the precise mechanisms are not well understood and need further investigation.

Unexpectedly, endophytic *Phomopsis* sp. made no obvious contributions to the grain yield of rice. Data analysis displayed no significant difference in grain yield between the endophyte-infected population and endophyte-free population. In *Epichloë bromicola*-grass association, the benefits of endophyte infection for the plant are coupled with the disadvantage of infertility (Groppe et al., 1999). This may be due to the different transmission model of the endophyte. The cryptic lifestyle of endophytic fungus and complex relationships between partners also probably influence the plant-fungal interaction. An endophyte that interacts mutualistically with its host plant may become pathogenic under certain stress conditions (Schulz et al., 2005). We presumed that the advantages conferred by the endophyte might be impaired at the final developmental stage of rice, undergoing natural leaf senescence. Moreover, the endophytic mycelium cultures were applied to the rice seedlings only once during the whole developmental stage, which probably made the endophytic inoculum inconsistently effective. Therefore, future studies should examine the effects of different volumes of endophytic inoculant or different periods of time on the grain yield.

In conclusion, endophytic *Phomopsis* sp. is horizontally transmitted to an extensive host range. This fungus is a mutualistic partner of non-host rice and alters the morphological and physiological properties of rice, promoting growth and increasing antioxidant enzyme levels and *Pn*. However, it does not affect the grain yield of rice. Understanding the physiological functions of *Phomopsis* sp may be useful in order to further exploit the endophyte's capacity to enhance the vigor and optimize the quality of plants; future research may suggest biotechnological applications of inducible fungal endophytes in green agriculture, horticulture, and forestry.

Acknowledgements

The authors are grateful for the support of the State Key Basic Research and Development Plan of China (No. 2005CB121107) and the Foundation of National Natural Science Foundation of China (No. 30500066).

REFERENCES

Arnold, A.E., Mejía, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., and Herre, E.A. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Science, USA* **100**: 15649–15654.

Bacon, C.W., Porter, J.K., Robbins, J.D., and Luttrell, E.S. 1977. *Epichloë typhina* from toxic tall fescue grasses. *Applied and Environmental Microbiology* **34**: 576–581.

Belesky, D.P., Devine, O.J., Pallas, J.E., Jr., and Stringer, W.C. 1987. Photosynthetic activity if tall fescue as influenced by a fungal endophyte. *Photosynthetica* **21**: 82–87.

Carroll, G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* **69**: 2–9.

Dai, C.C., Yu, B.Y., and Dong, C. 2005. Rapid propagation of medicinal plant *Euphorbia pekinensis*. *Guihaia* (in Chinese) **25**: 152–155.

Dorworth, C.E. and Callan, B.E. 1996. Manipulation of endophytic fungi to promote their utility as vegetation biocontrol agents. In: *Endophytic Fungi in Grasses and Woody Plants*. Reddin, S.C. and Carris, L.M., eds. APS Press, St. Paul, pp. 209–216.

Fisher, P.J. and Petrini, O. 1992. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). *New Phytologist* **122**: 137–143.

Giannopolitis, C.N. and Ries, S.K. 1977. Superoxide dismutase in higher plants. *Plant Physiology* **59**: 309–314.

Groppe, K., Steinger, T., Sanders, I., Schmid, B., Wiemken, A., and Boller, T. 1999. Interaction between the endophytic fungus *Epichloë bromicola* and the grass *Bromus erectus*: effects of endophyte infection, fungal concentration and environment on grass growth and flowering. *Molecular Ecology* **8**: 1827–1835.

Kranner, I., Cram, W.J., Zorn, M., Wornik, S., Yoshimura, I., Stabentheiner, E., and Pfeifhofer, H.W. 2005. Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. *Proceedings of the National Academy of Science, USA* **102**: 3141–3146.

Logan, B.A., Kornyeyev, D., Hardison, J., and Holaday, A.S. 2006. The role of antioxidant enzymes in photoprotection. *Photosynthesis Research* **88**: 119–132.

Madhava, R.K.V. and Sresty, T.V.S. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Science* **157**: 113–128.

Mucciarelli, M., Scannerini, S., Bertea, C., and Maffei, M. 2003. *In vitro* and *in vivo* peppermint (*Mentha piperita*) growth promotion by nonmycorrhizal fungal colonization. *New Phytologist* **158**: 579–591.

Murali, T.S., Suryanarayanan, T.S., and Geeta, R. 2006. Endophytic *Phomopsis* species: host range and implications for diversity estimates. *Canadian Journal of Microbiology* **52**: