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Effects of Phosphorus on Cadmium Tolerance and Accumulation in a Dark Septate Endophyte

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Abstract: *This study evaluated the effects of different phosphorus (P) concentrations on the growth of the dark septate endophyte (DSE)-Exophiala pinsciphila strain (ACCC 32496) under cadmium stress (100 mg L^{-1}). Also evaluated were the mineral nutrient contents (calcium, magnesium, nitrogen, phosphorus, and sulfur) and cadmium accumulation in the fungus. The mycelial mineral contents (Ca, Mg, S) and the culture media pH at low P concentrations (0.37-3.64 mmol L^{-1}) were higher than at high P conditions (18.35-36.7 mmol L^{-1}). The highest biomass, P content, and Cd content and accumulation in mycelium were obtained at high (18.35-36.7 mmol L^{-1}) P conditions. The mycelial biomass and Cd contents were significantly positively correlated with the mycelial P content. We conclude that high P concentrations improve the P content of DSE mycelium, enhance Cd tolerance in DSE, and increase Cd accumulation in mycelium.*

Keywords: *Phosphorus; Dark septal endophyte; Mineral nutrients; Cd tolerance*

1. Introduction

Dark septate endophytes (DSE) are polyphyletic ascomycetes found in the skin, cortex, and extracellular or intracellular vascular tissues in the roots of healthy plants. DSE mycelia are characterized by dark septate hyphae and microsclerotia [1]. DSE are widespread, have been observed in diverse harsh habitats such as deserts, tropical forests, cold zone mountains, and polar regions, particularly in cadmium (Cd) contaminated areas. In Cd polluted sites, DSEs widely exist in plant roots. Notably, DSEs exhibit high Cd tolerance [2].

The phosphorus (P) element is essential for biological growth, and can promote the biosynthesis of cell wall polysaccharide components. Besides, it is a biological antidote and a component of various biomolecules, such as proline (PRO), glutathione (GSH), phytochelatin (PCs), and non-protein thiols, among others. P participates in anti-oxidation reactions, organic acid metabolism, and resistance to Cd stress [3-5]. Therefore, this study used the DSE fungi *Exophiala pisciphila* to study the effects of different P concentrations on (i) the growth of DSE strains (ii) mineral element (Ca, Mg, N, P, S), and Cd contents, (iii) the Cd tolerance and accumulation of DSE strains. The findings of this study provide a theoretical basis for future research on the relationship between nutrient elements and fungal heavy metal tolerance.

2. Materials and methods

2.1. DSE strain and cultivation

The DSE fungi *E. pisciphila* ACCC 32496 was isolated from the roots of *Arundinella bengalensis* (*Poaceae*) growing naturally in an old mine-smelting site in Huize County, Yunnan Province, southwest China. The fungal strain is preserved in the Agricultural Culture Collection Center of China. We cultured the fungi in Modified Melin-Norkrans (MMN) liquid medium.

2.2. Experimental design

Exactly 1.015 g $CdCl_2.2.5H_2O$ was dissolved in a 5 L MMN medium [6] to a final Cd ion concentration of 100 mg/L. Three P concentrations of 0.37, 1.84, and 3.64 mmol L^{-1} in Cd-containing MMN medium were obtained by adding 0.3, 1.5, and 3.0 g KH_2PO_4 to three 900 mL of Cd-MMN mediums, respectively. The treatments with 18.35 and 36.7 mmol L^{-1} of P in Cd-MMN medium were obtained by adding 3.0 g KH_2PO_4 and 13.741 g NaH_2PO_4, and 3.0 g KH_2PO_4, and 30.89 g NaH_2PO_4 in

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two bottles of 900 mL Cd-MMN medium. Na^+ and K^+ were balanced with NaCl and KCl.900 mL of MMN medium was aliquoted into six 150 mL bottles and a single 250 mL flask. A square of the DSE strain (4-6 cm) was placed in each, and was cultured continuously for 7 days at 28℃, 120 min/r in a constant temperature shaker.

2.3. Indicator determination

Seven-day old cultures were filtered to obtain the hyphae as residue. The hyphae were then dried at 75℃ for 48 h, after which the biomass was determined by weighing and the number of spores evaluated using the blood cell counting method [7]. The mycelial contents of Cd, Ca, Mg, and S were determined by digesting with concentrated HNO_3-$HClO_4$, followed by flame atomic absorption spectrometry. The S content was determined by inductively coupled plasma mass spectrometry. Meanwhile, the N and P content in hyphae was determined from a concentrate prepared by H_2SO_4-H_2O_2 digestion, using the Nessler method and molybdenum antimony anti-colorimetric determination, respectively [8].

2.4. Data processing and statistical analyses

The data were collected and analyzed by Microsoft Excel, while figures were drawn using Origin 9.1. Significance testing (LSD method, significant level P=0.05) and variance analysis (Pearson method, significant level P=0.05) were done by IBM SPSS Statistics 21.

3. Results

3.1. Effects of different phosphorus concentrations on the biomass of DSE mycelium, the number of spores, and the culture pH

The P concentration in culture media significantly affected DSE mycelium biomass, the spore numbers, and culture media pH (Table 1). The mycelial biomass and the culture pH increased with an increase in the P concentration. The maximum mycelial biomass was obtained at a concentration of 18.35 mmol L^{-1} P, while the culture pH reached the maximum at 3.64 mmol L^{-1}P. The spore numbers at 1.84 and 18.35 mmol L^{-1} P were significantly higher than the numbers at 0.37, 3.64, and 36.7 mmol. L^{-1} P.

Table 1: Effects of different phosphorus concentrations on the biomass of DSE mycelium, the number of spores, and the culture pH

P concentration(mmol L^{-1})	Hyphae biomass(mg)	Spore number×10^3(N/ml)	pH value
0.37	119.67±4.51e	2.13±0.15bc	6.15±0.04c
1.84	184.00±10.82d	3.50±0.35a	6.72±0.05b
3.64	229.33±8.33c	1.93±0.40c	6.83±0.06a
18.35	406.57±15.24a	3.73±0.12a	6.10±0.03c
36.70	304.03±15.15b	2.53±0.12b	5.99±0.04d

Note: The different lowercase and uppercase letters in a column indicate significant differences among treatments at *P*<0.05 and *P*<0.01 levels, respectively.

3.2. Effects of different phosphorus concentrations on the mineral content in DSE mycelium

Table 2: Effects of different phosphorus concentrations in culture medium on mineral elements in DSE hyphae

P concentration(mmol L^{-1})	Ca Content (mg kg^{-1})	Mg Content (mg kg^{-1})	N Content (mg kg^{-1})	P Content (mg kg^{-1})	S Content (mg kg^{-1})
0.37	183±7bc	5693±93ab	730±110ab	4690±460d	1230±140b
1.84	193±2a	7643±566a	440±120c	6470±590c	1450±40a
3.64	171±9c	5408±591b	790±50a	7680±1580c	1430±50a
18.35	188±1b	5967±372ab	620±10b	15940±230b	1200±20b
36.70	148±3d	6412±670ab	800±90a	23970±190a	1140±60b

Note: The different lowercase and uppercase letters in a column indicate significant differences among treatments at P<0.05 and P<0.01 levels, respectively.

The P concentration in the culture solution significantly affected the mineral nutrient content of the mycelium (Table 2). At 0.37-3.64 mmol L^{-1} P, the mycelial Ca, Mg, and S contents were higher, and the mycelium Ca and Mg contents reached the maximum at 1.84 mmol L^{-1} P. The mycelium P content increased with the P concentration of culture, reaching the maximum at 36.7 mmol L^{-1} P.

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3.3. Effects of different phosphorus concentrations on cadmium content and accumulation in DSE mycelia

The mycelial Cd content and accumulation increased with the P concentration in the culture, reaching the maximum at 37.7 mmol L^{-1} P (Figure 1).

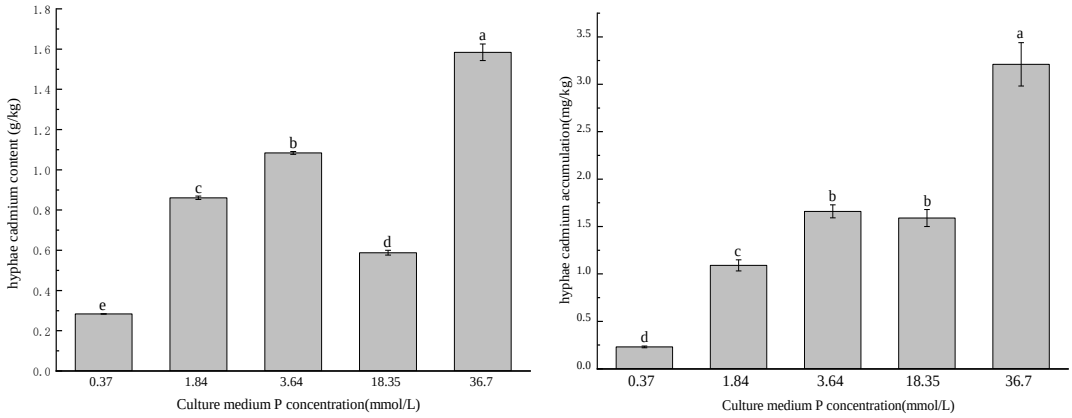

Figure 1: Effects of different phosphorus concentrations on cadmium content and accumulation in DSE mycelia

3.4. Correlation analysis

The mycelial Cd content was significantly negatively correlated with the mycelial Ca content, and significantly positively correlated with the mycelial P content. The mycelial biomass was significantly positively correlated with the mycelial P content. Meanwhile, the P content of the culture medium was extremely negatively correlated with the mycelial Ca and S contents, and exceptionally positively correlated with the mycelial P content. (Table 3).

Table 3: Correlation analysis between mycelial biomass, Cd content and mycelial mineral content

Item	Pearson correlation				
	Ca content	Mg content	N content	P content	S content
hyphae Cd content	-0.781**	0.164	0.301	0.677**	-0.057
Dry weight of hyphae	-0.181	-0.062	0.060	0.735**	-0.423
culture medium P content	-0.719**	0.026	0.336	0.992**	-0.667**

Note: * indicates significant correlation, ** indicates extremely significant correlation

4. Discussion

Growth status is an effective indicator of biological resistance in stressed environments. In this study conducted under Cd stress, a high concentration of P (18.35-36.7 mmol L^{-1}) resulted in the highest mycelial biomass and the lowest pH of the culture medium. This result could be because DSE secretes organic acids such as malic, tartaric, oxalic, and citric acids. Besides, DSE could produce protons through respiration and NH_4^+ assimilation, dissolving phosphate in the culture solution, thereby changing the culture media pH and promoting fungal growth [9].

The absorption of mineral elements by microorganisms is affected by the external environment and the interaction between mineral elements [10]. In this experiment, the mycelium Ca and S contents increased at low P concentrations (0.37-3.64 mmol L^{-1}), and decreased at high P concentrations (18.35-36.7 mmol L^{-1}). Thus, the mycelial P content increased with an increase in P concentrations. This result could be attributed to the dilution effect caused by increased mycelial biomass at high P concentrations. On the other hand, when large amounts of Ca, P, and S enter the cell, they form active groups that complex with heavy gold, causing DSE to absorb and accumulate high concentrations of Cd^{2+}. The high Cd^{2+} subsequently destroys the cell structure, disrupt cell ion channels, interrupt the anabolism of cellular substances, and affect nutrient absorption by DSE [11].

Heavy metal stress can change the mineral nutrient contents of fungal hyphae. Thus, applying mineral nutrient elements can alleviate the toxic effects of heavy metals on fungi [12]. There could be synergistic or antagonistic effects between heavy metal ions and multiple nutrient elements [13]. In this experiment,

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the mycelial Cd and Ca contents are significantly negatively correlated, and the two are significantly positively correlated to P content. These results could be because the ionic radius of Ca^{2+} is similar to Cd^{2+}. Therefore, Ca^{2+} and Cd^{2+}compete for ion channels into cell walls, cell membranes, and vacuolar membranes, generating an antagonistic effect [14]. P promotes DSE to synthesize numerous inorganic polyphosphate (polyP) molecules. Subsequently, the PolyP chelates with Cd^{2+}, increasing the mycelial Cd content [15]. However, linkage between phosphate and Cd-detoxifying mechanisms in E. pisciphila needs further study.

5. Conclusion

Under Cd stress, the mycelial Ca, Mg, and S contents, and the culture pH reached the maximum at low P concentrations (0.37-3.64 mmol L^{-1}). However, the DSE mycelium biomass, spore numbers, the mycelial N, P, and Cd contents, and the Cd accumulation were maximum at high P concentrations (18.35-36.7 mmol L^{-1}). This study further reports that the hyphal P content is significantly positively correlated with the mycelial biomass and Cd content. Therefore, 18.35-36.7 mmol L^{-1} P can promote P absorption by DSE, increase mycelial biomass, improve Cd tolerance, and increase mycelial Cd accumulation.

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