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RESEARCH ARTICLE

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Organic acid exudation from the roots of *Cunninghamia lanceolata* and *Pinus massoniana* seedlings under low phosphorus stress

Abstract Organic acid exudation from the roots of Chinese fir (*Cunninghamia lanceolata*) and Masson pine (*Pinus massoniana*) seedlings under low phosphorus stress was studied using the solution culture method. The results revealed that organic acid exudation from the roots of Chinese fir and Masson pine seedlings under low phosphorus stress increased. Compared with P_3 (KH_2PO_4, 0.5 mmol/L), the average organic acid exudation from the root of Masson pine seedlings under P_0 (KH_2PO_4, 0 mmol/L), P_1 (KH_2PO_4, 0.03 mmol/L) and P_2 (KH_2PO_4, 0.09 mmol/L) increased by 328.6%, 267.9% and 126.4% respectively. Masson pine from Zhejiang Province in China had the highest organic acid exudation. Under low phosphorus stress, the increase in organic acid exudation from the different provinces of Chinese fir and Masson pine varied. Masson pine from Zhejiang Province mainly increased oxalic acid, tartaric acid, citric acid and malic acid exudation, that from Guangxi Province mainly increased oxalic acid and tartaric acid exudation, and that from Guizhou Province, China mainly increased oxalic acid, tartaric acid and malic acid exudation. Chinese fir mainly increased oxalic acid and tartaric acid exudation.

Keywords low phosphorus stress, *Cunninghamia lanceolata*, *Pinus massoniana*, organic acids, root exudation

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1 Introduction

Phosphorus is one of the essential plant nutrient elements whose deficiency will heavily affect plant growth. It has been proven that some plants of different genotypes can excrete specific organic acids under normal levels of phosphorus. Rape mainly excretes citric acid and malic acid, while amaranth plants rich in potassium mainly excrete oxalic acid. The tips of non-row roots of white lupin (*Lupinus albus* L.) mainly secrete malic acid, acetic acid and fumaric acid, while row roots mainly excrete citric acid and malic acid (Ma, 1994; Chen et al., 2002; Li et al., 2005a, 2005b; Liang et al., 2005). Under low phosphorus stress or phosphorus deficiency, plants with high phosphorus efficiency often, by increasing organic acid exudation from roots, activate insoluble calcium-phosphorus, aluminum-bound phosphate and iron phosphate in the soil through acidification, complexation and chelation. The kinds and quantity of organic acids secreted by plant roots under low phosphorus stress reflect the adaptability of plants to P deficiency stress (Chen et al., 2002; Zeng et al., 2003; Xie et al., 2004). In recent years most researches on root exudates and organic acids exudation mainly related to herbs, rattans and small plants but was limited for trees. In this paper, organic acid exudation from the roots of Chinese fir (*Cunninghamia lanceolata*) and Masson pine (*Pinus massoniana*) seedlings under low phosphorus stress was studied using the solution culture method.

2 Materials and methods

2.1 Seedlings and experimental treatments

One-year-old seedlings cultivated with seeds from different zones were used as the experimental materials. Chinese fir included Longquan of Zhejiang Province (shortened as Chinese fir Zhejiang) and Fujian Province

(Chinese fir Fujian), while Masson pine included Linhai of Zhejiang Province (Zhejiang pine), Shangsi (Fangchenggang) of Guangxi Province (Guangxi pine) and Huishui of Guizhou Province (Guizhou pine).

Culture vessels with diameter of 10 cm and height of 20 cm were used to cultivate the seedlings; each vessel contained a 900 mL culture solution. Four seedlings were fixed on foam plastics in each vessel. The complete culture solution was referred to and adjusted from a Hoagland complete culture solution and formula recommended by Zhang (2003). Contents of the complete culture solution used in the experiment were as follows: KNO_3 1.5 mmol/L, KH_2PO_4 0.5 mmol/L, KCl 1.0 mmol/L, $Ca(NO_3)_2$ 2.0 mmol/L, NH_4NO_3 1.0 mmol/L, $MgSO_4$ 1.0×10^{-3} mmol/L, $ZnSO_4$ 1.0×10^{-3} mol/L, $CuSO_4$ 5.0×10^{-4} mmol/L, $(NH_4)_6Mo_7O_{24}$ 5.0×10^{-5} mmol/L, $MnSO_4$ 1.0×10^{-6} mmol/L, H_3BO_3 0.01 mmol/L and Fe-EDTA 0.1 mmol/L. P-deficiency culture solution was made by adjusting the concentration of KH_2PO_4 to 0 mmol/L (P_0), 0.03 mmol/L (P_1) and 0.09 mmol/L (P_2) respectively. Potassium chloride was added to make up for a loss of potassium ion, while other components of the P-deficiency culture solution remained the same as the complete culture solution. The complete culture solution was used as the normal P-support treatment (P_3: KH_2PO_4 0.5 mmol/L). All the tests had three replications. The acidity of the culture solution was adjusted to pH 4.5 ± 0.1 with diluted NaOH and HCl; the solution was totally changed every 3 days and subjected to continuous ventilation. From May 15, 2004 to July 5, 2004, the solution was used to cultivate seedlings. P-deficiency culture solution experiments were carried out from July 6, 2004 to July 15, 2004; three seedlings were kept in each vessel in the second period.

2.2 Collection of root organic acid exudation

Containers of 0.7 L used to collect root organic acids were cleaned and boiled with distilled water for 2 h ahead of the experiment. Collection steps were as follows: First, the seedling roots under P-deficiency stress were immersed in deionized water (a small amount of thymol was added to constrain the activity of microorganisms) for 5 min and washed three times to eliminate the remainder of the culture medium. Second, the roots were dipped in 0.05 mol/L $CaSO_4$ solution within the container (three drops of thymol added) from 8:00 to 12:00 (at constant ventilation), and the collection had three replications. Third, 600 mL of collected liquid was dried with a rotary evaporator under low temperature, then the solute (root exudation) was dissolved and diluted to 10 mL with 0.5% $(NH_4)_2HPO_4$ solution, after filtration through a micro-aperture filter membrane. The filtrate was detected with high performance liquid chromatography (HPLC) to reveal the kinds and quantity of organic acid exudation by different seedlings roots under different P-deficiency stress levels.

2.3 Determination of organic acids

The method of determining total organic acids was referred to Xie et al. (2004) and modified as follows: a 10 mL filtrate was diluted in 20 mL with distilled water and titrated to end point with 0.05% phenolphthalein as the indicator and 0.01 mol/L NaOH as the standard solution. There are three replications for this process. Total organic acid exudation by roots was calculated according to the consumption of sodium hydroxide.

The kinds and quantity of low-molecular-weight organic acids were determinated by HPLC. Measurement conditions were as follows: Hibar column RT 250 mm × 416 mm, made in Germany; diameter of filler 5 μm; temperature of column 30°C; mobile phase 0.5% $(NH_4)_2HPO_4$-H_3PO_4 buffer (pH 2.5, filtered with 0.22 μm membrane), flow velocity of 1 mL/min, UV detection wavelength of 214 nm, and injection volume 20 μL.

3 Results and discussion

3.1 Quantity of organic acids exudation by Chinese fir and Masson pine seedlings roots

With the decrease of phosphorus concentration in the culture solution, the quantity of organic acid excreted by Chinese fir and Masson pine roots increased gradually, but their variation ranges were different (Fig. 1). The phosphorus concentration of P_3 treatment was 5.5 times that of P_2, but there was only a minimal change in organic acid exudation between P_3 and P_2. While the phosphorus concentration of P_2 was three times that of P_1, the organic acid exudation between P_2 and P_1 changed significantly. This showed that with a decrease of concentration of phosphorus, the capability of organic acid exudation by Masson pine and Chinese fir roots was initially weak, subsequently strengthened and reached maximum at zero phosphorus level. But with the time of P-deficiency stress prolonged, exudation by the roots might gradually become reduced, eventually stopping excretion of organic acids as seedlings also stop growing. Further study is needed for the mechanism of the phenomenon. In terms of secreting organic acids under an all P-deficiency condition, Zhejiang pine had the highest capability of adaptation to low phosphorus stress among the tested trees.

3.2 Kinds of organic acids exuded by roots of Chinese fir and Masson pine

In normal P-support treatment (P_3), the Masson pine roots mainly secrete oxalic acid, tartaric acid and malic acid, while Chinese fir mainly secrete oxalic acid, tartaric acid, citric acid and malic acid (Table 1). Under P-deficiency stress, Masson pine and Chinese fir increased

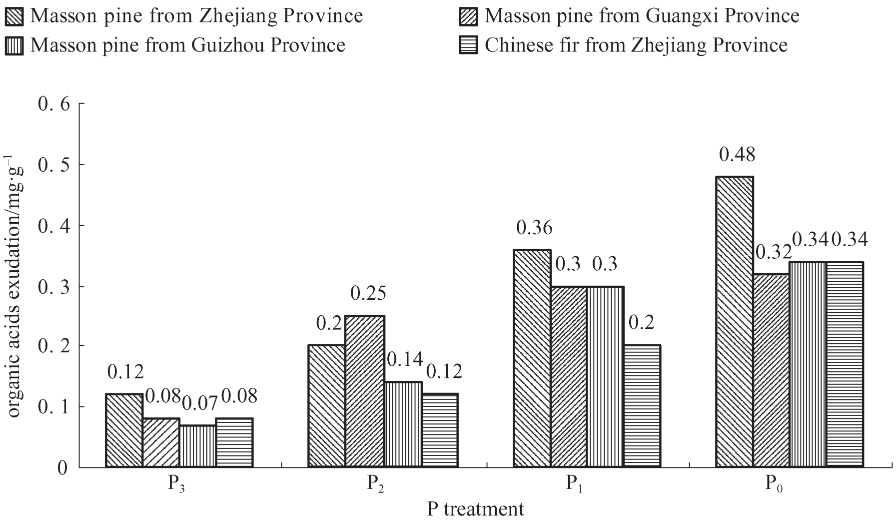

Fig. 1 Organic acids in root exudates of *Cunninghamia lanceolata* and *Pinus massoniana*

root organic acid exudation to some extent. Zhejiang pine mainly increased oxalic acid, tartaric acid, citric acid and malic acid exudation, while Guangxi pine mainly increased oxalic acid and tartaric acid exudation. Guizhou pine mainly promoted oxalic acid, tartaric acid and citric acid exudation, while Chinese fir mainly increased oxalic acid and tartaric acid exudation. There are big differences in organic acid exudation among the pine seedlings coming from different zones.

Under low phosphorus stress, increases in organic acid exudation from the different provinces of Chinese fir and Masson pine were different. In normal P-support treatment (P_3), oxalic acid and citric acid exudation by Zhejiang pine roots were undetected, whereas oxalic acid and citric acid exudation under low phosphorus stress (P_0) by Zhejiang pine roots reached 3.948 and 7.230 μg respectively. Tartaric acid secreted by Zhejiang pine under P_3 and P_0 treatment was 21.258 and 93,780 μg respectively. Malic acid exudation under P_0 treatment was 1.53 times that under normal P-support treatment (P_3). Oxalic acid and tartaric acid secreted by Guangxi pine roots under P_0 treatment was 3.26 and 3.62 times that under normal P-support treatment (P_3) respectively. Oxalic acid and citric acid secreted by Guizhou pine under P_0 treatment was 0.868 and 9.630 μg respectively; this secretion was undetected under normal P-support level (P_3). The oxalic acid and tartaric acid secreted by Chinese fir Zhejiang under P_0 treatment was 15.71 and 7.18 times that under normal P-support treatment (P_3). Oxalic acid exudation by Chinese fir Fujian was

Table 1 Kinds and amounts of organic acids in root exudates of *Cunninghamia lanceolata* and *Pinus massoniana* at different phosphor levels

	P level	pxalic acid /μg	tartaric acid /μg	citric acid /μg	malic acid /μg
Masson pine from Zhejiang Province	P_3	–	21.258	–	23.840
	P_2	0.381		–	–
	P_1	1.066	40.444	5.530	27.348
	P_0	3.948	93.780	7.230	36.604
Masson pine from Guangxi Province	P_3	0.327	22.255	–	–
	P_2	0.342	28.434	–	–
	P_1	1.293	53.462	–	–
	P_0	1.065	80.480	–	–
Masson pine from Guizhou Province	P_3	–	13.502	–	–
	P_2	0.434	13.981	4.320	–
	P_1	0.540	16.338	–	–
	P_0	0.868	17.243	9.630	–
Chinese fir from Zhejiang Province	P_3	0.133	8.762	4.574	5.304
	P_2	0.675	24.88	–	–
	P_1	1.350	26.650	–	–
	P_0	2.089	62.930	–	–
Chinese fir from Fujian Province	P_3	–	6.078	–	–
	P_2	0.543	13.584	–	–
	P_1	1.040	14.694	–	–
	P_0	1.850	16.676	–	–

undetected under normal P-support treatment (P_3), but reached 1.85 μg under P_0 treatment. Tartaric acid secreted by Chinese fir Fujian under P_0 treatment was 2.74 times that under normal P-support treatment (P_3).

Under P-deficiency stress, capability for oxalic acid and tartaric acid exudation by different pines decreased in the order Zhejiang pine, Guangxi pine and Guizhou pine. Oxalic acid and tartaric acid secreted by Chinese fir Zhejiang was greater than that of Chinese fir Fujian, There was more tartaric acid exudation than oxalic acid exudation for the Masson pine and Chinese fir roots under P-deficiency stress. The kinds and quantity of organic acid exudation by seedling roots depended on the mechanism of their physiological adaptation to the environment over a long time period.

4 Conclusions

1) Under P-deficiency stress, the quantity of organic acids excreted by roots of Chinese fir and Masson pine seedlings increased significantly. Compared with normal P-support treatment P_3 (KH_2PO_4, 0.5 mmol/L), the average quantity of organic acids excreted by roots of pine under the P-support treatment P_0 (KH_2PO_4 0 mmol/L), P_1 (KH_2PO_4 0.03 mmol/L) and P_2 (KH_2PO_4 0.09 mmol/L) increased by 328.6%, 267.9% and 126.4% respectively. Masson pine from Zhejiang has the highest capability of organic acid exudation among the tested pine seedlings. The organic acids excreted by Chinese fir roots under the P-support treatments P_0, P_1 and P_2 increased by 325.0%, 150.0% and 50.0% respectively compared to the normal P-support treatment. Organic acid exudation from the roots of Zhejiang fir was lower than that of Zhejiang pine.

2) In normal P-support treatment (P_3), the roots of Chinese fir and Masson pine can excrete low-molecular-weight organic acids to some extent. Chinese fir mainly excretes oxalic acid, tartaric acid, citric acid and malic acid, while Masson pine mainly excretes oxalic acid, tartaric acid and malic acid.

3) Under P-deficiency stress, roots of Chinese fir and Masson pine increased organic acid exudation. Seedlings from different zones increased different types of organic acid exudation. Zhejiang pine mainly increased oxalic acid, tartaric acid, citric acid and malic acid exudation, while Guangxi pine mainly had oxalic acid and tartaric acid exudation. Guizhou pine mainly had tartaric acid, malic acid and oxalic acid exudation, while Chinese fir mainly secreted oxalic acid and tartaric acid. Organic acid exudation by roots of Chinese fir and Masson pine increased when P-deficiency stress increased.

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