Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):572-577

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The organic acids in root exudates of oiltea and its role in mobilization of sparingly soluble phosphate in red soil

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ABSTRACT

In order to reveal the variation of organic acids in root exudates of oiltea camellia (Camellia oleifera Abel.) under different phosphorus (P) concentration and its role in mobilization of sparingly soluble phosphate and uptake of P. Sand culture, liquid collection and P solubility experiment was adopted to study the organic acid secretion of oiltea' roots and P mobilization in red soil and Ca-P, Al-P and Fe-P by the exudates and organic acids. The results showed that oiltea's seedling height, stem diameter, dried weight and P contents increased while root/shoot ratios decreased with the raise of P concentration in culture solution. Acetic acid and succinic acid are main acids secreted by oiltea, and oxalic, formic, malic and citric acids increased significantly in response to phosphorus deficiencies. Root exudates enhanced the mobilization of red soil plant-unavailable phosphorus by 100%-211%, and released P from Ca-P had no significant difference with water released, but which from Al-P and Fe-P were 0.2569 g/kg and 75.8 mg/kg higher than that. It was confirmed that there were significant difference in mobilization of Ca-P, Al-P and Fe-P by organic acids and the activation P values by oxalic, malic and citric acids were highest. The greatest extraction capability of P from red soil was obtained by citric acids, followed by oxalic and malic acids. These findings can help to understand the adapting mechanism of oiltea to low phosphorus environment, and provide a reference for management of P in cultivation.

Keywords: Acid soil, *Camellia oleifera*, P activation, Phosphorus deficiency

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INTRODUCTION

Phosphorus (P) is one of the major elements which are indispensible for plants' growth and development. However, it is hard for plants to take in enough P since it exists in the forms of sparingly soluble phosphate such as Fe-P, Al-P and Ca-P in acid red soil regions [1] and also because of the weak diffusivity of soluble phosphorus, P has become the main factor limiting the rise of crop yield in these regions [2]. Studies have shown that plants can adapt to the low P environment by boosting the release of sparingly soluble phosphate from the soil through many ways such as the secreting organic acids by roots [3, 4]. Low-molecular organic acids secretion induced by P deficiency including oxalic acid, malic acid and citric acid and so on, which can activate and promote plants' utility of sparingly soluble phosphate in the soil by chelating metal ions associated with P and reducing pH of rhizosphere [5], which has been verified in many crops such as rape (*Brassica napus* L.) [6], pigeonpea (*Cajanus cajan* L.) [7] and sugar beet[8]. Currently, it is a hot issue to promote mobilization of P in order to improve the utilization efficiency of phosphate fertilizer and reduce non-point source pollution of fertilizer, whereas studies on woody plants are relatively fewer.

Oiltea (*Camellia oleifera* Abel.), as an important woody oil tree species in China[9], its cultivated area has reached to 3.7 million hm^2 with the annual output of 300 million kg oil and average output of only 45～90 kg/hm^2. Such a low output and income seriously hinder the development of oiltea industry [10]. Oiltea is mainly planted in red acid soil regions in South China [11], so P deficiency is the major factor restricting the increase in output of oil yield

[12-13]. Up to now, a series of studies on P deficiency and P fertilizer in oiltea have been conducted [14-15], but whether sparingly soluble phosphate can be used by oiltea or not and the mechanism of how to use it are still uncertain. Aiming to provide references for explaining the adapting mechanism of oiltea to low P environment and management of P in cultivation, the kinds and amounts of organic acids in the root exudates of *Camellia oleifera* 'Xianglin XLC15' under different phosphorus concentration have been tested and the mobilization of P in red soil, Al-P, Fe-P and Ca-P by these organic acids has been explored in this paper.

EXPERIMENTAL SECTION

Material and Reagents

Camellia oleifera 'Xianglin XLC15' planted widely has been selected in this test. 1-year-old grafted seedlings with similar growth were selected in 5^{th} October, 2011. Their roots were trimmed before being washed with clean water and washed with distilled water before being disinfected with Carbendazim; after that they were planted in pots (with the diameter of 10 cm and height of 20 cm) of fine sands and cultivated in the open air but shielding from the rain with Hoagland-Arnon nutrient solution in a completely randomized design. Three treatments with different P concentrations (0, 0.1 and 1.0 mmol/l) were set and other elements were controlled unchanged in these treatments. P was provided by KH_2PO_4 and the shortage of K^+ in the nutrient solution was compensated by equivalent KCl; the solution's pH was adjusted to about 5.5 with 0.5% $Ca(OH)_2$ or H_2SO_4. 200 ml nutrient solution was sprinkled to each pot every 3 days and 100 ml distilled water was used at the evening to guarantee the water supply in hot weather. There were 5 replicates (3 individuals each) for each treatment.

Standards of formic acid, acetic acid, malic acid, succinic acid, fumaric acid and tartaric acid were bought from Dr.Ehrenstorfer; citric acid, malonic acid, lactic acid standards were bought from SIGMA. $AlPO_4$, $Ca_3(PO4)_2$ and $FePO_4 \cdot 2H_2O$ were bought from Kermel Chemical Reagent Co., LTD and they were expressed as Al-P, Ca-P and Fe-P respectively. Red soils used in exudates dissolution test were collected from Central South University of Forestry and Technology, Changsha (28°8′14″N, 112°59′32″E, 149 m in altitude) and from Shuangqiao Village, Li County (29°50'7" N, 111°43'12" E and 71 m in altitude) and South Tower ridge, Suxian District (25°46'56" N,113°01'58" E and 270 m in altitude), Hunan province, China; the red soil used in the organic acid dissolution test was collected from oiltea base in Huangtang village, Changning county, Hunan province (26°23′44″N, 112°35′56″ E and 207 m in altitude). Red soil was grounded manually and passed through a mesh sieve (2 mm) after air drying.

Measurements of plant growth and P contents

Seedling height and stem diameter at ground level were measured after roots exudates being collected, and then the seedlings were harvested and divided into roots and shoots. Dried weight (DW) and roots/shoot ratio (DW of root/DW of shoot) after dried at 65°C for 24 h were obtained. Dried old leaves (about 7 weeks old), new leaves (less than 7 weeks old) and roots were grinded and digested with H_2SO_4+H_2O_2 (EasyDigest 40, AMS, Italy). P content was measured by the same method in dissolution test.

Collection and Analysis of Root Exudates

The plants were got out from their pots carefully on 20^{th} June, 2012 and then washed clean with small water flow. Their roots were bathed in deionized water 5 minutes and washed for 3 times, and then put the each replicate into a wide-mouthed black container with 250 ml 0.05 g/l $CaSO_4$. During the collecting process, the solution was ventilated constantly for 24 hours (from the morning to the next morning 8:00). Then the $CaSO_4$ solution was concentrated on the Rotary Evaporator (temperature was controlled at 45°C; revolution was 80- 90 r/min) to 50 ml, and concentrated solution of root exudates from different replicates were pooled within each treatment before being reserved in the refrigerator (-20°C). 2-3 drops of 0.05% thymol were used in the bath, collecting and reserving to control the activities of microorganisms.

Common low-molecular organic acids secreted by plant roots [8, 16] such as oxalic, citric, tartaric, malic, succinic, acetic, malonic, formic, lactic and fumaric acids were mixed to prepare the mixed standard solution. Fig.1 is the chromatogram of the standard mixture. HPLC-high performance liquid chromatograph (A700, Agilent Technologies, America) was used to measure the kinds and concentration of organic acid in the root exudates with external standard method and measurement of peak area respectively. The determination condition: ZORBAX SB-C18(5 μm, 250 mm×4.6 mm); mobile phase was: 0.01mol g/l KH_2PO_4(PH=2.73)：CH_3OH=98:2; wavelength was 215 nm; flow velocity was 1 ml/min; column temperature was 25°C and the sample size was 20 μl.

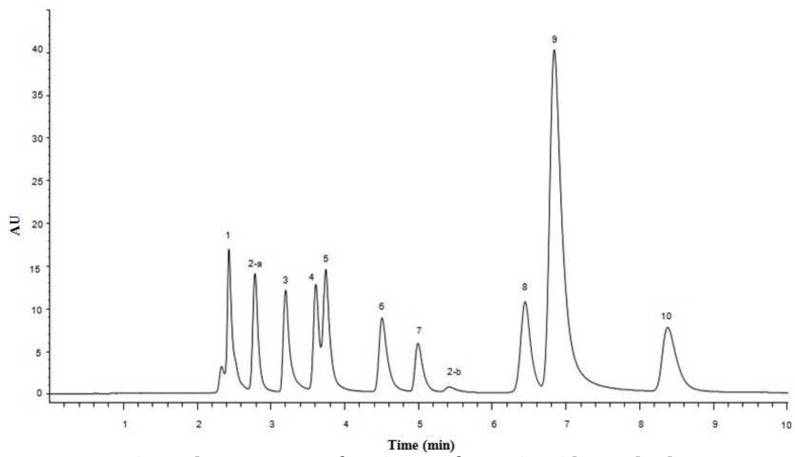

Fig.1. Chromatogram of a mixture of organic acids standards

1. Oxalic acid; 2. 2-a and 2-b: tartaric acid; 3. Formic acid; 4. Malic acid; 5. Malonic acid; 6. Lactic acid; 7. Acetic acid; 8. Citric acid; 9. Fumaric acid; 10. Succinic acid

Mobilization of P in Soil and Sparingly soluble phosphate

Chemical reagents of 2 main organic acids in the root exudates (acetic acid and succinic acid) and 4 others (oxalic acid, formic acid, malic acid and citric acid) which were significantly induced by low P of oiltea were used to prepare 1.0 mmol/l solutions separately and concentrated solution of root exudates were returned to room temperature before the test on mobilization of P in soil and sparingly soluble phosphate.

Exudates dissolution test: put 20 ml concentrated root exudates and 0.1g Fe-P, 0.1g Al-P, 0.1g Ca-P or 5.0 g soil in a 50 ml plastic bottle and fasten the bottle cap; Organic dissolution test: put 20 ml organic acid solution and 0.1g Fe-P, 0.1g Al-P, 0.1g Ca-P or 5.0 g soil in a 50 ml plastic bottle and fasten the bottle cap. then put them on the thermostatic oscillator for 6-hour oscillation (200 r/min, 25°C). The comparison group was treated with water extraction.

The extracting solution was centrifuged for 10 minutes with the speed of 3000 r/min in a 10 ml centrifuge tube. P concentration in supernatant and root exudates was measured(Smartchem 200, Westco Scientific Instruments, Italy) by the molybdenum-ascorbic acid method[17]. Then mobilization of P by root exudates was calculated by subtracting the P concentration of root exudates from supernatant.

Statistical Analysis

All collected data were subjected to one-way ANOVA followed by Duncan's multiple range tests for mean comparisons. All analysis was performed using SPSS for Windows, V17.0. Figures and tables were drawn with Origin 8.6 and Excel 2003.

RESULTS

Plant growth and P Contents

There were significant differences in seedling height, stem diameter, dried weight, root/shoot ratio and P content in oiltea with different P concentrations (Fig.2 and Table 1). With the rise of P concentration in culture solutions, oiltea's seedling height, stem diameter and dried height was increased; when the P concentration was 1.0 mmol/l, the dried weight of shoot was 3.18 times as that of shoot in the P-free culture solution, and the dried weight of root was significantly higher than that by no P and 0.1 mmol/l P cultivation, though there were no significant difference between next two cases. The relative increase of root growth leads to the decrease of root/shoot ratio with the decrease of P concentration, which is the typical symptom showing plants' adaption to low P environment. And it has been found out P contents in different parts of oiltea increased with the increase of P concentration and the ranking of P contents was root>new leave>old leave. In old leaves, P contents (0.79 g/kg) treated by 1.0 mmol/l P were significantly higher than that treated by 0 mmol/l and 0.1 mmol/l P concentration, whose values were 0. 66 and 0.67 g/kg respectively. P contents in new leaves increased with the increase of P concentration in culture solutions: 0.76 g/kg, 0.90 g/kg and 1.25 g/kg respectively, while P content in root reached the peak-3.90 g/kg when the concentration was 1.0 mmol/l, which was 4.6 times and 3.6 times as the two former cases.

Table 1: Morphological differentiation of oiltea under different P concentration

P concentration (mmol/l)	Seedling height (cm)	Stem diameter (cm)	Dried weight of shoot (g)	Dried weight of root (g)	Root/shoot ratio
0.0	38.57 b	0.66 b	3.24 c	4.30 b	1.33 a
0.1	45.50 a	0.72 b	5.02 b	4.01 b	0.79 b
1.0	46.40 a	0.84 a	10.30 a	6.02 a	0.59 c

Different letters indicated significant difference at P<0.05

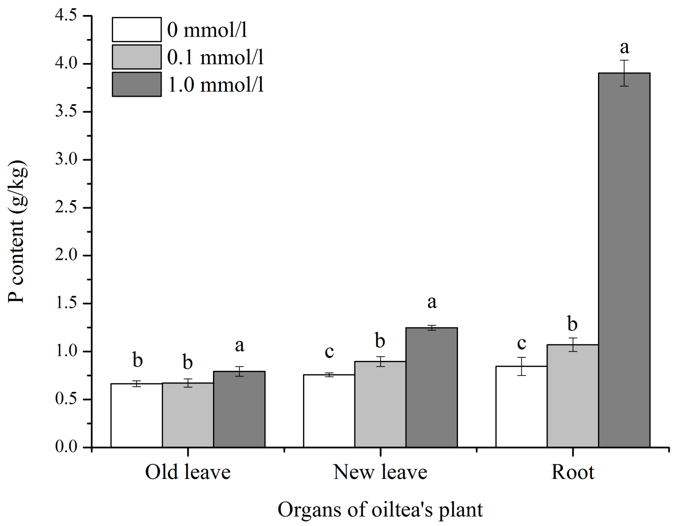

Fig.2. P content of oiltea under different P concentration

Different letters above or below standard error bars indicated significant difference at P<0.05

Organic acids in root exudates

Ten organic acids in the root exudates of oiltea were measured by HPLC, the results showed (Table 2) that the concentration of these 10 organic acids in root exudates were significantly different in oiltea under different P concentration and secretion of organic acids were improved by low P. In respect of the amounts, acetic acid and succinic acid ranked the first; formic acid and tartaric acid the second and fumaric acid the last. Except for the fumaric acid, 9 other organic acids were secreted more than those secreted under the condition of no P and low P concentration (0.1 mmol/l), and the secreted amounts of oxalic acid, formic acid, citric acid and malic acid were the maximum under the condition of no P while the secreted mounts of tartaric acid, malonic acid, lactic acid, acetic acid and succinic acid were the maximum when the P concentration was 0.1 mmol/l.

Table 2.Organic acid from root exudation of oiltea under different P concentration

P concentration (mmol/l)	Oxalic acid (mg/l)	Tartaric acid (mg/l)	Formic acid (mg/l)	Malic acid (mg/l)	Malonic acid (mg/l)	Lactic acid (mg/l)	Acetic acid (mg/l)	Citric acid (mg/l)	Fumaric acid (mg/l)	Succinic acid (mg/l)
0	10.35a	11.604 b	30.371 a	6.808 a	5.860 b	5.323 b	129.568 b	5.794 a	0.045 a	51.010 b
0.1	6.582 b	33.484 a	29.582 a	4.256 a	16.110 a	145.273 a	341.853 a	5.716 a	0.046 a	75.112 a
1.0	5.035 c	2.134 c	13.989 b	1.094 b	1.639 b	4.281 b	89.096 b	4.984 b	0.042 a	35.719 b

Different letters indicated significant difference at P<0.05.

Mobilization of P in soil and sparingly soluble phosphate by root exudates

Root exudates collected by solution method were employed to extract P in soil and sparingly soluble phosphate. Results (Fig.3 and Fig.4) showed that these exudates can obviously solubilize soil P and sparingly soluble phosphate. Soil P extraction test revealed that the dissolving of soil P by exudates in Changsha, Changde and Chenzhou was remarkably higher than that by water (P<0.05) and P concentration extracted in these three samples were 100%, 157% and 211% higher than that by water respectively. Sparingly soluble P dissolution test revealed that Ca-P and Al-P are easier to be dissolved than Fe-P. Though the mobilization of Ca-P by root exudates was numerically weaker than that by water, there was no significant difference (P=0.078>0.05), while mobilization of Al-P and Fe-P by root exudates were stronger than that by water (P=0.005，P=0.030) and the dissolved P were 0.2569 g/kg and 75.8 mg/kg higher than that by water respectively.

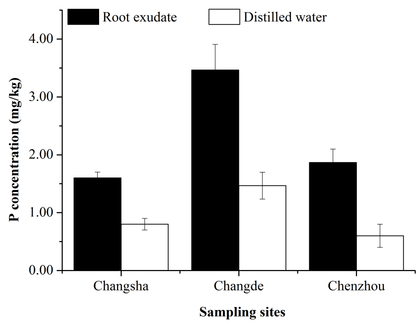

Fig.3.Mobilization of soil P by root exudates of oiltea
Different letters above or below standard error bars indicated significant difference at P<0.05

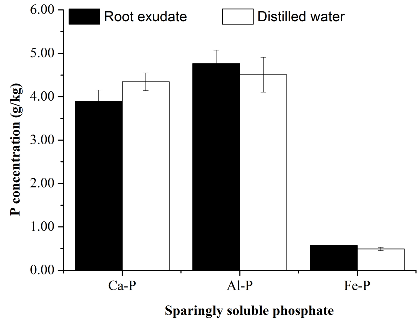

Fig.4.Mobilization of Ca-P, Al-P and Fe-P by root exudates of oiltea
Different letters above or below standard error bars indicated significant difference at P<0.05

Mobilization of P by organic acids

Results above indicate that low P induced excessive secretion of some organic acids and the secretion can activate sparingly soluble P. In order to verify the role of organic acids in mobilization of sparingly soluble P, chemical reagents of 2 main organic acids and 4 others induced by low P significantly were selected for simulation analysis of P mobilization of Fe-P, Al-P and Ca-P and red soil by organic acids secreted by oiltea roots. Results (Table 3) showed that the greatest extraction capability of P from red soil was obtained by citric acid, followed by oxalic acid and malic acid and that of formic acid was the weakest. However, P extraction capabilities of acetic acid and succinic acid whose amounts were the most in root exudates were just 70% and 40% of that of citric acid respectively. Solubility experiment of sparingly soluble P by organic acids found that the mobilization of Fe-P ranked the first by oxalic acid, followed by citric acid and by malic acid where there was no significance difference, while that by acetic acid and by succinic acid was the weakest. The mobilization of Al-P by citric acid, oxalic acid and malic acid was higher than that by formic acid and acetic acid which was higher than that by succinic acid-about 30% of that by citric acid. Extraction capability of Ca-P by oxalic acid was the greatest, followed by citric acid, malic acid, formic acid and succinic acid.

Table 3.Mobilization of soil P, Ca-P, Al-P and Fe-P by organic acids

Organic acids	Red soil (mg/kg)	Fe-P (mg/kg)	Al-P (mg/kg)	Ca-P (mg/kg)
Acetic acid	0.15 c	-	363.44 b	519.67 c
Succinic acid	0.29 b	-	207.65 c	162.41 e
Citric acid	0.39 a	63.80 b	683.28 a	590.65 b
Oxalic acid	0.37 ab	224.31 a	728.47 a	636.24 a
Malic acid	0.38 ab	59.57 b	720.33 a	580.44 b
Formic acid	-	11.88 c	330.28 b	229.98 d

Different letters indicated significant difference at P<0.05

DISCUSSION

Most of the phosphate exists in the forms of sparingly soluble phosphate such as Fe-P, Al-P and Ca-P in acid red soil[18] where oiltea is widely planted, therefore, available P concentration in soil is too low for its growth. It is of great significance to study how oiltea can get enough P and the role of its root exudates in P mobilization in order to reveal adopting mechanism of low-P and improve the management of P in cultivation of oiltea. This study demonstrated that oiltea's seedling height, stem diameter, dried weight and P nutrient decrease while root/shoot ration increase after P stress, which is in line with findings of previous studies[19]. Under the condition of low P, kinds and amounts of organic acids secreted by plants changed[16, 20], our works found that secretion amounts of organic acids are noticeably higher in P-free and low P cultures than those in high P culture and 4 acids (oxalic acid, fromic acid, malic acid and citric acid) can be influenced significantly by low P induction, which coincides with findings in other plants[6-8]. The amount of Acetic acid is the most in oiltea, which is the same with that in masson pine[21] but a little different from that in carrots[22], which may be caused by the difference between woody and herbaceous plants. Within 2 hours, plants are free from the damage of being drown in solution and root exudates are not be degraded obviously by the microorganism activities [23]. Hence, in spite of ventilation has been used in this study, a further study on differentiation of root exudates of oiltea by different collection ways will be helpful in future studies.

The availability of P in soil can be improved by organic acids because of its hydroxyl and carboxyl chelate with P-fixing ions like Fe^{2+}, Al^{3+} and Ca^{2+}, the absorption reaction between negative ions which can release P from sparingly soluble medium, and solubilize sparingly soluble P in soil by release H^+ to rhizosphere [5, 8, 24-25]. This study found that the root exudates of oiltea can activate P in soil and sparingly soluble P where organic acids play an important role and the roles of citric acid, oxalic acid and malic acid are the most remarkable, which indicated that different solubility of P in soils by root exudates is due to different composition of sparingly soluble phosphate and different P solubility of organic acids. Although organic acids' role in mobilization of sparingly soluble phosphate has been verified in this paper, test on mobilization of sparingly soluble phosphate by root exudates under different P concentration has not been conducted because of small amounts collected. Therefore, the mobilization of P by root exudates of oiltea under different P concentration and the composition of other secretions and their role in mobilization of P in soil require further study.

CONCLUSION

Under the condition of low P, oiltea's seedling height, stem diameter and dried weight decrease and plant is short of P nutrition, while the root/shoot ratio will increase; the main organic acids in the root exudates are acetic acid and succinic acid and oxalic, formic, malic and citric acids which are induced obviously by low P; the absorption of P by oiltea can be improved through secreting organic acids which can raise the concentration of available P. The work can provide reference to understand the adapting mechanism of oiltea to low phosphorus in acid red soil and for the management of P in cultivation.

Acknowledgement
This work was funded by the Hunan Major Projects of Science and Technology (2013FJ1006).

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