INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19F–150/2020/23–5–1033–1043
DOI: 10.17957/IJAB/15.1383
http://www.fspublishers.org

Full Length Article

Effects of Different Phosphorus Supply Levels on Organic Acid Secretion in *Hydrilla verticillata* Roots in Plateau Wetland

Yangyi Zhao, Xu Duan* and Jiancong Zhang
College of Ecology and Environment, Southwest Forestry University, Kunming 650224, China
*For correspondence: feixue20012360@163.com
Received 26 August 2019; Accepted 18 December 2019; Published 03 March 2020

Abstract

The eutrophication of wetland waters is becoming a serious concern, and the self-purification capacity of wetlands is gradually decreasing. This study used a nutrient solution hydroponic method to determine the levels of organic acids secreted by the typical submerged plant *Hydrilla verticillata* in the plateau wetland under phosphorus stress of 0, 0.2, 1, 5, 10, and 20 mg/L by gas chromatography-mass spectrometry (GC-MS). The relative contents and variation in the nine organic acids secreted by the *Hydrilla* roots at 7, 14, 21 and 28 days after culture were analyzed. The results showed that the relative contents of phthalic acid and phenolic carbonate secreted by *H. verticillata* roots were significantly higher than those of other organic acids during the same phosphorus stress period. When the duration of phosphorus stress was 7 days, the secretion of pyrocarbonic acid, phthalic acid, benzenedicarboxylic acid, benzoic acid, and total organic acid was minimal at 0 mg/L phosphorus concentration, and the secretion of rock carbonic acid increased with the increase in phosphorus concentration. The secretion of benzenedicarboxylic acid initially decreased and then increased while the secretion of benzoic acid and phthalic acid initially increased and then decreased. The secretion of oxalic acid decreased with the increase in phosphorus concentration. Phthalic acid, benzenedicarboxylic acid, and benzoic acid have a very significant positive correlation with phosphorus stress concentration. With the prolongation of stress time, the secretion of phthalic acid, benzenedicarboxylic acid, and total organic acid was the lowest. Under no phosphorus stress, the concentration of sulfurous acid, carboxylic acid, sulfonic acid, benzenedicarboxylic acid, and phosphorus had a significant positive correlation ($r = 0.865$, $r = 0.864$, $r = 0.919$, and $r = 0.589$). In conclusion, the *H. verticillata* root system primarily regulates the secretion of phthalic acid under phosphorus stress, which is an important mechanism to adapt to environmental changes. The results of this study can further expand our understanding regarding eutrophication and micro-control measures in plateau wetlands.

Keywords: Phosphorus; Organic acid; Root exudates; *Hydrilla verticillata*

Introduction

A wetland has strong ecological purification ability. In recent years, the eutrophication of wetland water is on the rise (Tang *et al.* 2010; Hou *et al.* 2018; Zou *et al.* 2018), with phosphorus being a main factor (Yu *et al.* 2010; Qin *et al.* 2013; Zhou *et al.* 2016; Roy 2017), which wetland plants effectively absorb and utilize. The root system is the key bridge between wetland plants and the survival medium; therefore, to investigate energy circulation (Akhtar *et al.* 2016; You *et al.* 2013; Zhang *et al.* 2018a; b), it is essential to study root system exudates.

Studies have shown that organic acids secreted by roots can regulate rhizosphere micronutrients and change the structural and physicochemical properties of root microbes in a plant's growth environment. Organic acids can also regulate plant resistance to adverse environments and relieve hypoxia; therefore, studying them aids in the understanding of the mechanism of plant resistance (Hinsinger 2001; Kuang *et al.* 2003; Rellan-Alvarez *et al.* 2010; Zhao *et al.* 2016). The effects of phosphorus stress on the secretion of organic acids by plant roots have been investigated. For example, rice roots excrete more organic acids under low phosphorus stress (Li *et al.* 2005; Deng *et al.* 2006; Wang *et al.* 2014); while some plants induce phosphorus release from soil by changing root morphology and increasing secretion of organic acids in a phosphorus-deficient environment (Zhou *et al.* 2011; Ma *et al.* 2017). Low molecular weight organic acids in root exudates of wheat and broad bean could improve the bioavailability of phosphorus. In addition, Deng *et al.* (2006) studied the effect of phosphorus concentration on root exudates of *Pinus yunnanensis* seedlings, demonstrating the changes in organic acids in the exudates of *P. yunnanensis* seedlings. The root exudates of wetland plants primarily consisted of small molecular organic acids and aromatic proteins (Lu *et*

To cite this paper: Zhao Y, X Duan, J Zhang (2020). Effects of different phosphorus supply levels on organic acid secretion in *Hydrilla verticillata* roots in plateau wetland. *Intl J Agric Biol* 23:1033–1043

al. 2009; Huang *et al.* 2014). Previous studies have focused on the effects of low phosphorus stress on cash crops and trees, as well as root exudation and rhizosphere effects of wetland plants. Although the effects of plant root exudates and phosphorus stress on plant roots have been investigated, there are few studies regarding the response mechanism in root exudates of submerged plants to phosphorus stress as well as the effect of phosphorus stress on organic acid secretion. Therefore, this study aimed to understand the stress response of root exudates to pollution and to explain the mechanism of phytoremediation.

Hydrilla verticillata is a submerged herb. Some studies have shown that *Hydrilla* can transform phosphorus from wetland sediment to be utilized, and it can purify the water after eutrophication (Zhao *et al.* 2008; Li *et al.* 2016).

Materials and Methods

Water culture and solution ratio

The Hoagland nutrient solution was used in the laboratory of Southwest Forestry University at the beginning of March to culture *Hydrilla* seedlings at 20–30°C. Phosphorus stress was induced at the beginning of April, and was measured at 7, 14, 21, and 28 days. Eighty plastic barrels were used as hydroponic equipment, where the Hoagland nutrient solution was added. Healthy *Hydrilla*, 160, with insignificant differences in seedling age were selected and 2 seedlings were cultured in each barrel. In the barrels containing the plants, a 300 mm rubber disc microporous aerator was installed to prevent the plant root system from rotting by ensuring a sufficient amount of aeration. To avoid the algal blooming in the barrel, aluminum foil was used for shading (Horchani *et al.* 2008). The culture medium was changed once a week. After one month of culture, *Hydrilla* plants with good growth and similar height were selected and transplanted into the experimental device. Monopotassium phosphate (KH_2PO_4) was used as the phosphorus source, with a phosphorus concentration gradient of 0, 0.2, 1, 5, 10, and 20 mg•L^{-1}. There were 72 plastic barrels (4 groups of experiments, 3 replicates in each group), and 2 plants were cultured in each barrel.

Hoagland nutrient solution contained: potassium sulfate (K_2SO_4): 0.75×10^{-3} mol•L^{-1}, magnesium sulfate ($MgSO_4$): 0.75×10^{-3} mol•L^{-1}, potassium chloride (KCl): 1×10^{-3} mol•L^{-1}, calcium nitrate ($Ca(NO_3)_2$): 2.0×10^{-3} mol•L^{-1}, Boric acid (H_3BO_3): 1×10^{-5} mol•L^{-1}, copper(II) sulfate ($CuSO_4$): 1×10^{-7} mol•L^{-1}, manganese(II) sulfate ($MnSO_4$): 1×10^{-6} mol•L^{-1}, zinc sulfate ($ZnSO_4$): 1×10^{-6} mol•L^{-1}, ammonium heptamolybdate ($(NH_4)6Mo_7O_{24}$): 5×10^{-6} mol•L^{-1}, Fe-EDTA ($C_{10}H_{12}FeN_2O_8$): 1×10^{-4} mol•L^{-1}. This stock was 4× diluted for the hydroponic culture (Chen, 2009).

Collection and isolation of root exudates

The entire root system of the plant cultured under phosphorus stress was washed with deionized water for 7, 14, 21, and 28 days, and root exudates were collected, (collection solution ratio: 5 μmol•L^{-1} H_3BO_3, 600 μmol•L^{-1} calcium chloride ($CaCl_2$), 100 μmol•L^{-1} KCl, and 200 μmol•L^{-1} magnesium chloride ($MgCl_2$), pH 5.6). After repeated washing for three times, the root system was covered with a black plastic bag, and transferred to a beaker containing 50 mL root exudates. The root exudates were collected under natural light for 4 h (9:00–13:00). The root lotion was obtained by transplanting the root in solution containing 1 L of 0.5 mmol L^{-1} $CaCl_2$ for 4 h (13:00–17:00) (Tian *et al.* 2003; Zhang *et al.* 2007). The extraction solution was obtained using dichloromethane (CH_2Cl_2) extraction and root lotion for three times (40 mL/times). Finally, 200 mL extraction solution was collected at 38℃, dried with anhydrous (Na_2SO_4), and then concentrated to dry reserve by vacuum rotary evaporator (Wei *et al.* 2016).

Determination of root exudates

The solution was extracted with a syringe after a full shake of CH_2Cl_2, which was added to the rotating evaporation at 1.2 knots for 0.5 mL, after passing through a 0.45 μm needle filtration membrane. Meanwhile, the membrane was filtered using a 0.45 μm needle and then put into a small brown bottle for further GC-MS analysis. The organic acids in root exudates were determined by gas chromatography/mass spectrometry (Agilent 7890B). The chromatographic conditions were as follows: the capillary column was HP-5 ms column (30 m × 250 μm × 0.25 μm) (Huiyong *et al.* 2013; Liu *et al.* 2017), the injection port temperature was 260°C, the carrier gas was helium (He) (purity of 99.999%), the flow rate was 1 mL min^{-1} with injection volume of 1 μL, the flow valve was opened after 1 min, the column temperature was programmed, starting at 50°C, with flow rate of 2 min; 20°C per minute, programmed to 150°C, and 5°C per minute, programmed to 220°C, then, 6°C per minute, programmed to 250°C, for 15 min.

As for the mass spectrum conditions (Liu *et al.* 2017), electron bombardment source (EI) ionization energy was 70eV, ion source temperature was 200°C while the interface temperature was 280°C and quadrupole temperature was 150°C, solution delay time was for 3.75 min, scanning mode (SCAN) with a scanning range of M/Z 33-453, and standard tuning.

Data analysis

The organic acids in *Hydrilla* root exudates were identified by artificial analysis of total ion flow map and referencing it with the standard map of NIST08 mass spectrometry database. The determination of root exudates was carried out by computer search. The relative concentration of substances was calculated according to the peak area (%) of the components which is detected in the chromatogram.

Data processing and statistical methods

In this study, excel WPS2016 and SPSS21 software were used for data processing and statistical analysis. The LSD method was used for multiple comparisons. The significant level, α, was 0.05, while the extremely significant level α was 0.01, and the scanning map was drawn by origin8.5.

Results

The organic acids and relative contents in root exudates of *H. verticillata* were determined under different phosphorus concentration gradient stress by artificially analyzing the GC-MS scanning map. Six different phosphorus stress conditions were used and the results were referenced with the standard map of NIST08 mass spectrometry database. The organic acids secreted in response to six concentrations of phosphorus stress and four time periods were analyzed, and were classified into the total organic acids.

GC-MS scanning pattern of root exudates from *H. verticillata*

In this paper, only the scanning of four different time periods, at phosphorus concentration of 20 mg•L^{-1} is listed. The results of scanning chart 1 show that the scanning spectra are different at different time periods, and the number of characteristic peaks as well as the areas of high and low peaks are not consistent. Each characteristic peak represents a compound, and thus scanning spectra indicates that the secretion of root exudates under phosphorus stress differs depending on the time periods.

Differences in organic acids secretion in the roots of *H. verticillata* at different phosphorus concentrations and stress periods

Changes in organic acid secretion under different phosphorus concentrations during the same stress period: Table 1 shows that when phosphorus stress time was at 7 days, the secretion of carbonic acid, phthalic acid, phenyldicarboxylic acid, benzoic acid and total organic acid was the lowest (phosphorus concentration is 0 mg/L), with relative content of: 0.11, 3.09, 0.51, 0.01 and 7.85%, respectively. Under a concentration of 20 mg/L phosphorus, the exudation of stone carbonate and phenyldicarboxylic acid was the highest. No significant differences were observed in secretion at phosphorus concentration of 10 mg/L and 20 mg/L. The secretion of benzodicarboxylic acid initially decreased then increased. There was no significant difference in secretion between 0 mg/L and 0.2 mg/L phosphorus concentration. The highest secretion of benzoic acid and phthalic acid was observed when phosphorus concentration was 10 mg/L, and then decrease as phosphorus concentration increased. There was no significant difference in benzoic acid secretion between 1, 10 and 20 mg/L phosphorus concentration. The maximum secretion of total organic acids was observed when the concentration of phosphorus was 1 mg/L. The lowest secretion of sulfurous acid and carboxylic acid was observed when phosphorus concentration was 10 mg/L. The secretion at 0–10 mg/L phosphorus concentration decreased significantly in the range concentration of sulfite. The secretion at 20 mg/L phosphorus concentration was significantly higher than that of the 10 mg/L. There was no significant difference in carboxylic acid between 5, 10 and 20 mg/L phosphorus concentration, in which the minimum secretion of carboxylic acid was observed at 0.2 mg/L phosphorus concentration. Oxalic acid secretion gradually decreased as the phosphorus concentration increased. The maximum amount of sulfonic acid was observed when concentration of phosphorus was 0 mg/L while the minimum amount was observed at 5 mg/L.

When phosphorus stress lasted for 14 days, the highest amount of excretion was obtained from phthalic acid at 36.45%, phenyldicarboxylic acid at 4.20%, sulfonic acid at 0.59%, and total organic acid at 65.88% under phosphorus concentration of 1 mg/L. The contents of phthalic acid and phthalic acid was the least when phosphorus concentration was 0 mg/L. There was no significant difference between the two acids in the relative content at 10 and 20 mg/L phosphorus concentration; additionally, in the range of 1–10 mg/L phosphorus concentration, the content of the two acids decreased significantly. The secretion of sulfonic acid and total organic acid of 1 mg/L was significantly higher than that of 0 mg/L. The lowest secretion of sulfonic acid and total organic acid was 0.23 and 16.12% under 5 mg/L phosphorus concentration respectively, in the 1–20 mg/L region, the secretion decreased significantly under 20 mg/L phosphorus concentration. The secretion of carbonates, carboxylic acids and benzoic acid was the lowest when phosphorus concentration was 0 mg/L, and the order of relative content was: 0.32, 0.41 and 0.22%, respectively. While the concentration of carboxylic acid and benzoic acid at 1 mg/L phosphorus was higher than that of 0 mg/L, there was no significant difference in the secretion of carboxylic acid between 0.2, 10, and 20 mg/L. There was no significant difference in the content of benzoic acid under phosphorus concentrations between 1, 10, 5, and 20 mg/L. The maximum secretion of carbonic acid was 17.43%, when the concentration of phosphorus was 0.2 mg/L, and the amount of excretion between 0 and 20 mg/L decreased significantly. The maximum amount of sulfite excretion was 1.06% in the 20 mg/L phosphorus concentration treatment, and at least 0.26% in 0.2 mg/L phosphorus concentration treatment, while the amount of secretion between 1 and 20 mg/L increased gradually. The maximum excretion of oxalic acid, 0.59%, was observed in the 0.2 mg/L phosphorus concentration treatment, and at least 0.21% in the 5 mg/L phosphorus concentration treatment. There was no significant difference in the secretion of sulfurous acid between 5, 1, and 20 mg/L phosphorus concentration treatments.

Table 1: Comparison of relative content of organic acids in root exudates of *H. Verticillata* under different phosphorus concentration and time treatment (%)

Organic acid	Concentration/(mg/L)	Duress time/(day)			
		7	14	21	28
Sulfurous acid	0	1.15 ± 0.03 a-A	0.44 ± 0.04 c-C	0.49 ± 0.06 b-BC	0.56 ± 0.04 d-B
	0.2	0.94 ± 0.03 b-B	0.26 ± 0.06 d-D	0.46 ± 0.04 b-C	1.11 ± 0.04 b-A
	1	0.59 ± 0.05 d-B	0.44 ± 0.03 c-B	0.40 ± 0.08 b-B	1.37± 0.14 a-A
	5	0.47 ± 0.04 e-B	0.87 ± 0.06 b-B	0.46 ± 0.05 b-C	1.06 ± 0.10 b-A
	10	0.37 ± 0.07 f-B	0.94 ± 0.07 b-A	0.90 ± 0.04 a-A	0.60 ± 0.09 cd-B
	20	0.80 ± 0.06 c-BC	1.06 ± 0.05 a-A	0.88 ± 0.05 a-B	0.74 ± 0.08 c-C
Phenol	0	0.11 ± 0.02 e-C	0.32 ± 0.02 f-B	0.87 ± 0.05 e-A	0.34 ± 0.04 d-B
	0.2	1.03 ± 0.15 d-C	17.43 ± 0.08 a-A	9.68 ± 0.08 c-B	0.53 ± 0.06 c-D
	1	6.25 ± 0.06 c-C	12.65 ± 0.05 c-A	9.51 ± 0.03 d-B	0.62 ± 0.05 b-D
	5	6.28 ± 0.06 bc-C	16.64 ± 0.07 b-A	10.14 ± 0.07 b-B	0.79 ± 0.03 a-D
	10	6.49 ± 0.04 a-B	1.69 ± 0.11 d-C	9.60 ± 0.06 cd-A	0.33 ± 0.02 d-D
	20	6.53 ± 0.05 a-B	1.38 ± 0.10 e-C	10.26 ± 0.06 a-A	0.20 ± 0.01 e-D
Carboxylic Acid	0	0.54 ± 0.06 c-A	0.41 ± 0.03 c-B	0.31 ± 0.04 c-C	0.56 ± 0.02 f-A
	0.2	1.26 ± 0.05 a-B	0.77 ± 0.06 b-C	0.28 ± 0.04 c-D	2.01 ± 0.03 a-A
	1	0.72 ± 0.10 b-C	0.86 ± 0.07 ab-B	0.12 ± 0.02 d-D	1.95 ± 0.05 b-A
	5	0.38 ± 0.04 d-C	0.92 ± 0.05 a-B	0.45 ± 0.05 b-C	1.27 ± 0.04 d-A
	10	0.34 ± 0.02 d-C	0.76 ± 0.09 b-B	1.13 ± 0.12 a-A	0.94 ± 0.03 e-D
	20	0.39 ± 0.04 d-D	0.71 ± 0.03 b-C	1.06 ± 0.10 a-B	1.76 ± 0.02 c-A
Phthalic acid	0	3.09 ± 0.02 c-B	3.96 ± 0.07 d-A	3.98 ± 0.17 b-A	0.36 ± 0.01 c-B
	0.2	3.52 ± 0.11 c-B	27.67 ± 1.04 b-A	27.10 ± 0.17 a-A	0.97 ± 0.05 a-B
	1	28.20 ± 1.80 b-B	36.45 ± 1.03 a-A	26.23 ± 1.49 a-B	0.43 ± 0.02 bc-C
	5	29.73 ± 1.54 ab-A	9.92 ± 0.26 c-C	27.01 ± 1.05 a-B	0.49 ± 0.04 b-D
	10	30.84 ± 1.51 a-A	6.90 ± 1.05 d-B	3.38 ± 0.22 b-C	0.34 ± 0.05 c-D
	20	29.90 ± 1.57 ab-A	7.84 ± 0.96 d-B	0.51 ± 0.03 c-C	0.37 ± 0.03 c-C
Oxalic acid	0	0.70 ± 0.09 a-A	0.41 ± 0.08 b-B	0.55 ± 0.11 a-B	0.06 ± 0.01 d-C
	0.2	0.57 ± 0.09 b-A	0.59 ± 0.06 a-A	0.52 ± 0.04 b-A	0.43 ± 0.04 b-B
	1	0.49 ± 0.05 bc-AB	0.33 ± 0.05 bc-B	0.39 ± 0.10 c-B	0.56 ± 0.03 a-A
	5	0.39 ± 0.03 c-B	0.21 ± 0.04 c-C	0.25 ± 0.03 d-C	0.53 ± 0.03 a-A
	10	0.30 ± 0.04 cd-B	0.44 ± 0.05 b-A	0.50 ± 0.05 bc-A	0.23 ± 0.04 c-B
	20	0.28 ± 0.04 d-B	0.27 ± 0.04 c-B	0.67 ± 0.06 a-A	0.18 ± 0.03 c-C
Benzene dicarboxylic acid	0	0.51 ± 0.04 e-B	0.60 ± 0.09 e-B	0.78 ± 0.06 f-A	0.11 ± 0.02 b-C
	0.2	0.54 ± 0.05 e-C	3.19 ± 0.08 b-A	2.93 ± 0.05 e-B	0.23 ± 0.03 a-D
	1	3.62 ± 0.04 b-B	4.20 ± 0.07 a-A	3.07 ± 0.02 d-C	0.09 ± 0.02 b-D
	5	3.48 ± 0.09 c-A	1.19 ± 0.02 c-C	3.32 ± 0.04 c-B	0.08 ± 0.01 bc-D
	10	3.26 ± 0.09 d-B	0.76 ± 0.10 d-C	3.45 ± 0.09 b-A	0.05 ± 0.01 c-D
	20	3.74 ± 0.05 a-A	0.88 ± 0.07 d-B	3.69 ± 0.12 a-A	0.03 ± 0.00 c-C
Benzoic Acid	0	0.01 ± 0.00 d-D	0.22 ± 0.02 d-C	0.78 ± 0.05 d-B	0.89 ± 0.09 d-A
	0.2	0.22 ± 0.01 c-D	1.12 ± 0.11 b-B	1.78 ± 0.05 b-A	0.99 ± 0.05 d-C
	1	0.97 ± 0.10 a-C	1.28 ± 0.06 a-B	1.96 ± 0.06 a-A	1.26 ± 0.04 c-B
	5	0.71 ± 0.04 b-D	0.77 ± 0.06 c-C	1.47 ± 0.05 c-B	2.11 ± 0.09 b-A
	10	1.04 ± 0.04 a-C	1.37 ± 0.08 a-B	0.28 ± 0.06 f-D	3.85 ± 0.22 a-A
	20	1.00 ± 0.18 a-A	0.88 ± 0.04 c-A	0.49 ± 0.05 e-B	0.23 ± 0.04 e-C
Sulfonic acid	0	0.58 ± 0.02 a-A	0.32 ± 0.03 b-B	0.09 ± 0.03 f-D	0.23 ± 0.05 e-C
	0.2	0.16 ± 0.02 d-D	0.52 ± 0.04 a-B	0.31 ± 0.03 e-C	1.84 ± 0.05 a-A
	1	0.18 ± 0.02 cd-C	0.59 ± 0.04 a-B	0.59 ± 0.05 d-B	1.57 ± 0.06 b-A
	5	0.22 ± 0.03 c-B	0.23 ± 0.04 c-B	0.68 ± 0.04 c-A	0.30 ± 0.06 e-B
	10	0.28 ± 0.03 b-D	0.53 ± 0.05 a-C	1.12 ± 0.05 b-B	1.27 ± 0.08 c-A
	20	0.31 ± 0.03 b-B	0.31 ± 0.03 b-B	1.34 ± 0.06 a-A	0.92 ± 0.03 d-C
Total organic acid	0	7.85 ± 0.21 c-A	6.68 ± 0.19 f-B	7.85 ± 0.37 d-A	3.11 ± 0.05 d-C
	0.2	8.54 ± 0.20 c-C	55.13 ± 1.45 b-A	49.60 ± 0.10 a-B	9.86 ± 0.28 b-C
	1	49.48 ± 1.80 a-B	65.88 ± 1.17 a-A	48.64 ± 1.41 a-B	9.95 ± 0.30 b-C
	5	46.29 ± 1.43 b-B	32.95 ± 0.47 c-C	49.55 ± 1.06 a-A	8.29 ± 0.15 c-D
	10	47.86 ± 1.62 ab-A	18.89 ± 1.76 d-C	22.75 ± 0.46 b-B	11.90 ± 0.23 a-D
	20	47.62 ± 1.68 ab-A	16.12 ± 1.14 e-C	19.90 ± 0.27 c-B	9.63 ± 0.05 b-D

Note: the figures in the table were all repeated mean ±standard errors in three groups. The lowercase letters after the numbers in the table indicate the differences (longitudinally) in secretions between different concentrations of phosphorus in the same organic acids at the same time of stress, The uppercase letters represent the differences (horizontal) between different stress time treatments of the same organic acid at the same phosphorus concentration. In the same column (or row), the same letter means that the difference is not significant ($p > 0.05$), while the letter difference means the difference is significant

When phosphorus stress duration was 21 days, the highest secretion of phenyldicarboxylic acid, sulfonic acid and oxalic acid appeared at the stress of phosphorus concentration of 20 mg/L, and the relative content was as follows: 3.69, 1.34 and 0.67%, respectively. Both phthalic acid and sulfonic acid as well as oxalic acid excretions increased significantly with the increase in phosphorus concentration, with no significant difference between 0.2 and 10 mg/L phosphorus concentration in the secretion of oxalic acid. The minimum amount of excretion was 0.25%

at phosphorus concentration 5 mg/L. The maximum exudation of carbonic acid, phthalic acid and total organic acid were all at the concentration of 0.2 mg/L, while the minimum secretion of carbonated stone and total organic acid was both observed in the 0 mg/L phosphorus concentration, and the minimum secretion of phthalic acid was 0.32% in the 0 mg/L phosphorus concentration. There was no significant difference between total organic acid and phthalic acid under the phosphorus concentrations of 0.2, 1, and 5 mg/L, but the secretion under 0.2 mg/L was significantly higher than that under 0 mg/L. The secretion of carbonic acid increased significantly under 0–5 mg/L; the maximum amount of sulfurous acid and carboxylic acid secreted was at 10 mg/L, and the least amount of acid excretion observed in the 1 mg/L phosphorus treatment. There was no significant difference between the two acids at the concentration of 10 and 20 mg/L, and 0 and 0.2 mg/L. The maximum secretion of benzoic acid was 1.96% where phosphorus concentration was 1 mg/L, and the minimum secretion of benzoic acid was 0.28% when phosphorus concentration was 10 mg/L. The secretion of benzoic acid increased significantly in 0–1 mg/L. There was a significant decrease in secretion between 5 and 20 mg/L.

The maximum secretion of carboxylic acid, phthalic acid, benzoic acid, and sulfonic acid appeared at 28 days of phosphorus stress, when phosphorus concentration was 0.2 mg/L, and the relative content of carboxylic acid, phthalic acid, benzoic acid, and sulfonic acid in turn was 2.01, 0.97, 0.23 and 1.84%, respectively. The secretion of above four acids in 0.2 mg/L was significantly higher than that in 0 mg/L. In the range of 0.2–20 mg/L concentration, the excretion of phthalic acid decreased with the increase in phosphorus concentration. There was no significant difference in secretion between 0, 1, 10, and 20 mg/L, and no significant difference in the relative content of phthalic acid and benzene dicarbonic acid in the 1 and 5 mg/L phosphorus concentration treatments. The secretion of sulfonic acid in response to 0.2–5 mg/L phosphorus concentration was significantly decreased; the content of benzoic acid and total organic acid was the highest in the 10 mg/L phosphorus concentration treatment. The content of benzoic acid in 20 mg/L was significantly larger than that in 10 mg/L, and the amount of benzoic acid in response to 0–10 mg/L increased with the increase in phosphorus concentration. However, there was no significant difference in secretion between 0 and 0.2 mg/L. The secretion of sulfurous acid and oxalic acid was the highest when phosphorus concentration was 1 mg/L, and it was the lowest at the 0 mg/L. The secretion of the two acids in the 0–1 mg/L region was significantly increased, but there was no significant difference in secretion at the phosphorus concentrations of 10 and 20 mg/L. The secretion of sulfurous acid in the 1–10 mg/L region decreased significantly, so did the oxalic acid in the 1–20 mg/L region. As phosphorus concentration increased, the secretion gradually decreased. The secretion of carbonaceous acid increased significantly in the 0–5 mg/L concentration and decreased significantly in the 5–20 mg/L concentration.

Changes in organic acid secretion in different time periods under the same phosphorus concentration stress

Table 1 shows that when phosphorus concentration was 0 mg/L, the maximum secretion of sulfurous acid, oxalic acid, sulfonic acid and total organic acid was observed at 7 days, while the minimum secretion of oxalic acid and total organic acid was at 28 days. The excretion of oxalic acid decreased gradually with the increase in culture time, but there was no significant difference in the secretion of total organic acid between 7 and 21 days. The minimum secretion of sulfite was 0.44% after 14 days of culture, and there was no significant difference between 14, 21, and 28 days, but the amount of secretion gradually decreased between 7 and 28 days. The secretion of sulfonic acid and phthalic acid increased significantly between 7 and 21 days, and the secretion of 28 days was significantly lower than that of 21 days. While the maximum secretion of carboxylic acid and benzoic acid was observed at 28 days, the secretion of carboxylic acid decreased significantly from 7 to 21 days, and the secretion of benzoic acid increased with the increase in culture time. The maximum secretion of phthalic acid and benzene dicarbonic acid was 21 days, and the secretion of phthalic acid increased significantly between 7 and 21 days, but there was no significant difference between 14 and 21 days of culture period. The minimum secretion of benzene dicarbonic acid was 0.11% at 28 days, and there was no significant difference between 7 and 14 days.

When phosphorus concentration was 0.2 mg/L, the secretion of sulfurous acid, carboxylic acid and sulfonic acid was the highest at 28 days, the relative content was 1.11, 2.01 and 1.84%, respectively. The secretion of sulfurous acid and carboxylic acid decreased significantly in the range of 7–21 days. The secretion at 28 days was significantly larger than that of 21 days, while the minimum secretion of sulfonic acid was 0.16% at 7 days, the secretion at 28 days was significantly higher than that of the other three periods. The maximum exudation of carbonate, phthalic acid, total organic acid, and benzene dicarbonic acid were at 14 days, the secretion of all four acids in 14 days was significantly greater than that at 7 days. The secretion of phthalic acid and benzene dicarbonic acid decreased from 21 to 28 days, and the secretion of lithic acid at 28 days was significantly lower than that of the other three periods. The secretion of total organic acid decreased significantly from 14 to 21 days; the secretion of oxalic acid decreased gradually with the increase in culture time, and there was no significant difference between 7, 14, and 21 days. The secretion of benzoic acid increased significantly from 7 to 21 days, and the relative content of benzoic acid at 28 days was significantly lower than that of 21 days.

When phosphorus concentration was 1 mg/L, the secretion of sulfurous acid, carboxylic acid, oxalic acid, and

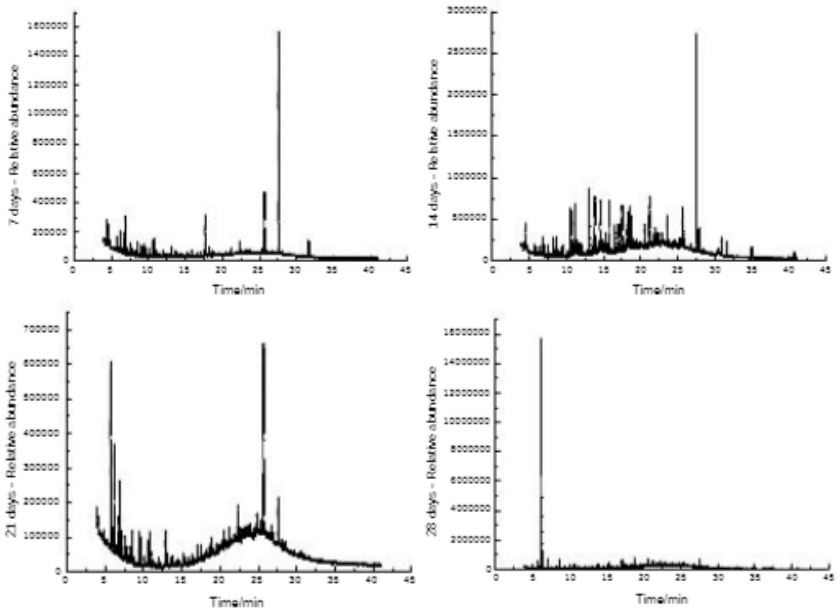

Fig. 1: Scanning map of root exudates of *H. Verticillata* at different times of 20 mg/L phosphorus concentration

sulfonic acid was the highest when cultured for 28 days and the relative content is shown in Table 1, Fig. 1. The secretion of sulfurous acid in 28 days was significantly higher than that in the other three periods. There was no significant difference in the relative content of sulfonic acid between 7, 14, and 21 days. The secretion of sulfonic acid increased significantly with the increase in culture time, and the secretion of carboxylic acid at 28 days was significantly higher than that in other periods; there was no significant difference in the secretion of oxalic acid at 7, 14, and 21 days. The total organic acid, phthalic acid, total organic acid, and phenyldicarboxylic acid secretions were the highest at 14 days and the lowest at 28 days. The secretion of three kinds of acids and total organic acids in 14 days were significantly higher than those in 7 days, and the secretion at 14 to 28 days decreased significantly. There was no significant difference in the secretion of phthalic acid between 7 and 21 days. The secretion of benzoic acid increased significantly in 7–21 days and decreased significantly in 28 days.

The specific relative content of each acid when phosphorus concentration was 5 mg/L is shown in Table 1. The secretion of sulfurous acid, carboxylic acid, oxalic acid, and benzoic acid appears at most in 28 days, the relative content in turn is 1.06, 1.27, 0.53 and 2.11%, respectively. Oxalic acid, sulfurous acid, and carboxylic acid showed the same regularity of decreasing and then increasing with the change of culture time. The secretion of oxalic acid at 28 days was significantly higher than that at other three periods, and there was no significant difference between 14 and 21 days of oxalic acid secretion. The secretion of benzoic acid increased with the increase in culture time. The amount of sulfonic acid and total organic acid was the highest at 21 days, but the secretion of sulfonic acid on days 7, 14, and 21 had no significant difference, and the total organic acid content at day 28 was significantly lower than that at the other three periods. The maximum secretion of phthalic acid and benzene dicarbonic acid was observed at 7 days and least at 28 days. With the increase in culture time, the secretion of stony carbonic acid decreased first and then decreased, and the secretion of carbonate at 14 days was significantly higher than that at 7 days, and the secretion from 14 to 28 days decreased significantly.

When phosphorus concentration was 10 mg/L, the maximum secretion of carbonated acid, carboxylic acid, oxalic acid, and phenyldicarboxylic acid was at 21 days, the minimum secretion of stone carbonate, oxalic acid, and phenyldicarboxylic acid was at 21 days, and the minimum secretion of carboxylic acid was at 7 days. The secretion of carboxylic acid and oxalic acid increased significantly from 7 to 21 days, and the secretion at 28 days was significantly lower than that at 21 days. Secretion of phthalene dicarboxylic acid and carbonic acid changed with the duration of culture and then increased and then decreased significantly. The specific relative content is shown in Table 1. The highest secretion of benzoic acid and sulfonic acid was observed at 28 days, the minimum secretion of benzoic acid was at 21 days, and the amount of sulfonic acid increased significantly with the increase in culture time. Both phthalic acid and total organic acid showed a significant decrease with the increase in culture time.

When phosphorus concentration was 20 mg/L, the secretion of phthalic acid, phenyldicarboxylic acid, benzoic acid, and total organic acid was the highest at 7 days and the lowest at 28 days. The relative content is shown in Table 1. The secretion of phthalic acid and total organic acid

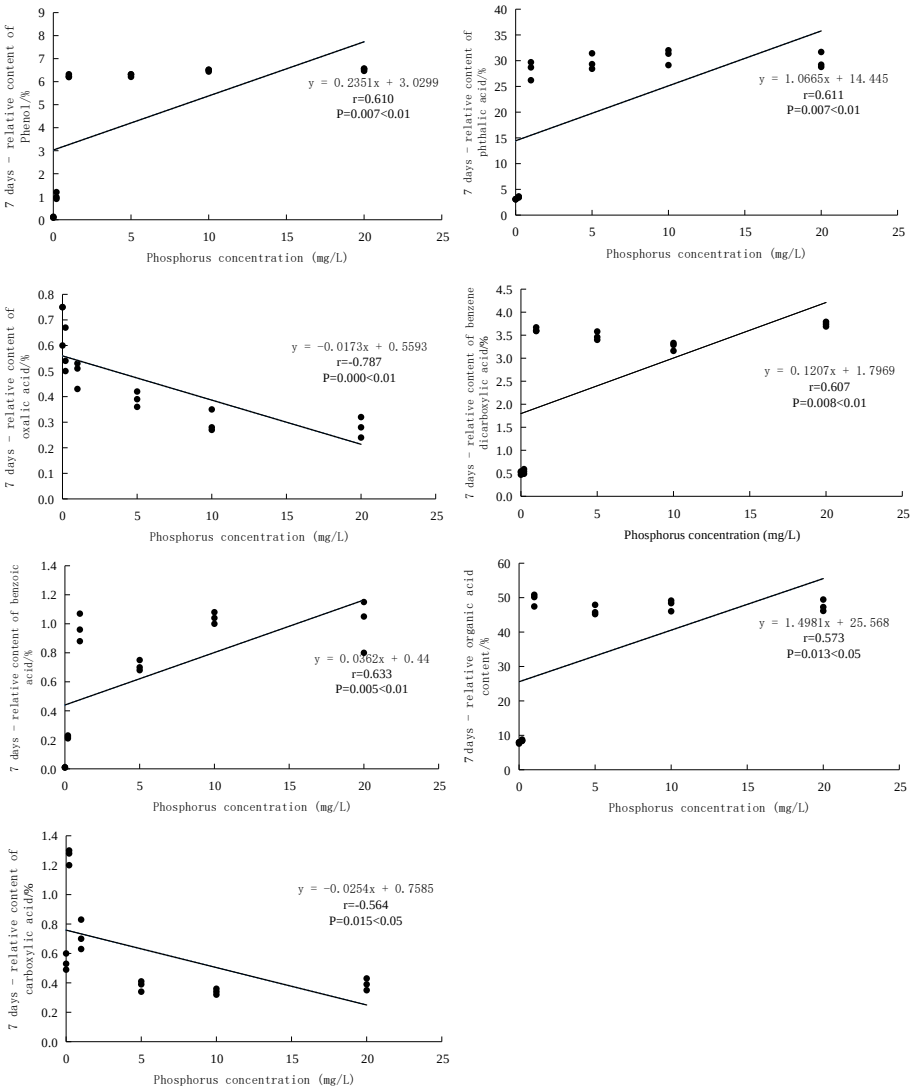

Fig. 2: Correlation analysis between phosphorus concentration and organic acid at 7 days of stress duration

increased significantly with the increase in culture time, and the secretion of benzoic acid increased gradually with the increase in culture time. There was no significant difference in the secretion of benzoic acid at 7 and 14 days. There was no significant difference in the secretion of benzene dicarbonic acid between 7 and 21 days. The secretion of stony carbonate and oxalic acid increased significantly from 7 to 21 days being the highest at 21 days and lowest at 28 days. The secretion of sulfurous acid initially increased followed by a decrease, and the secretion volume decreased significantly from 14 to 21 days. Carboxylic acid secretion increased significantly with the increase in stress duration.

Correlation analysis between phosphorus concentration and relative content of organic acids in roots

By correlation analysis, we found a significant positive correlation between the concentrations of carbonic acid, phthalic acid, phenyldicarboxylic acid and benzoic acid in root exudates of *H. verticillata* cultured for 7 days (Fig. 2); ($r = 0.610$, $P = 0.007$; $r = 0.611$, $P = 0.007$; $r = 0.607$, $P = 0.008$; $r = 0.633$, $P = 0.005$). The results showed that with the increase in phosphorus stress concentration, the secretion of carbonic acid, phthalic acid, phenyldicarboxylic acid and benzoic acid also increased, and the total organic acid had a significant positive correlation with phosphorus stress concentration ($r = 0.573$, $P = 0.013$), and significant negative correlation between oxalic acid and phosphorus stress concentration ($r = -0.787$, $P = 0.000$). The relative content of oxalic acid decreased as the phosphorus concentration increased, additionally, there is a significant negative correlation between carboxylic acid and phosphorus stress concentration, ($r = -0.564$, $P = 0.015$), There was no significant correlation between sulfurous acid

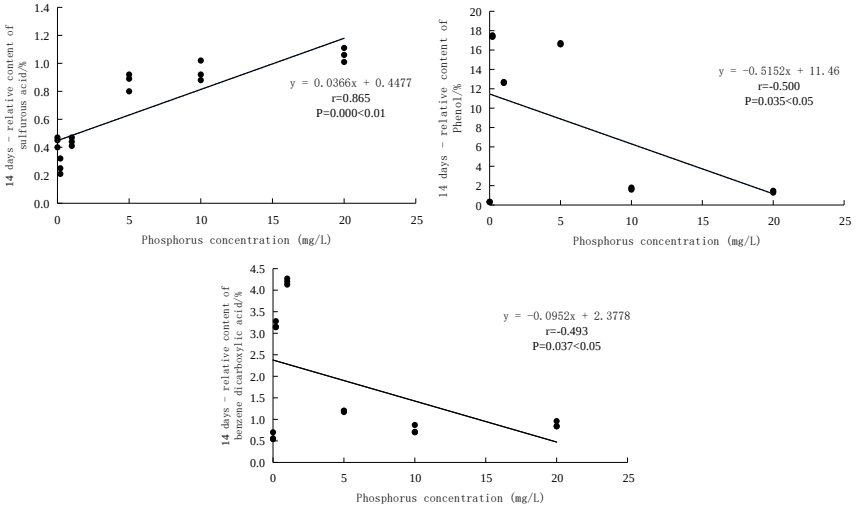

Fig. 3: Correlation analysis between phosphorus concentration and organic acid at 14 days of stress duration

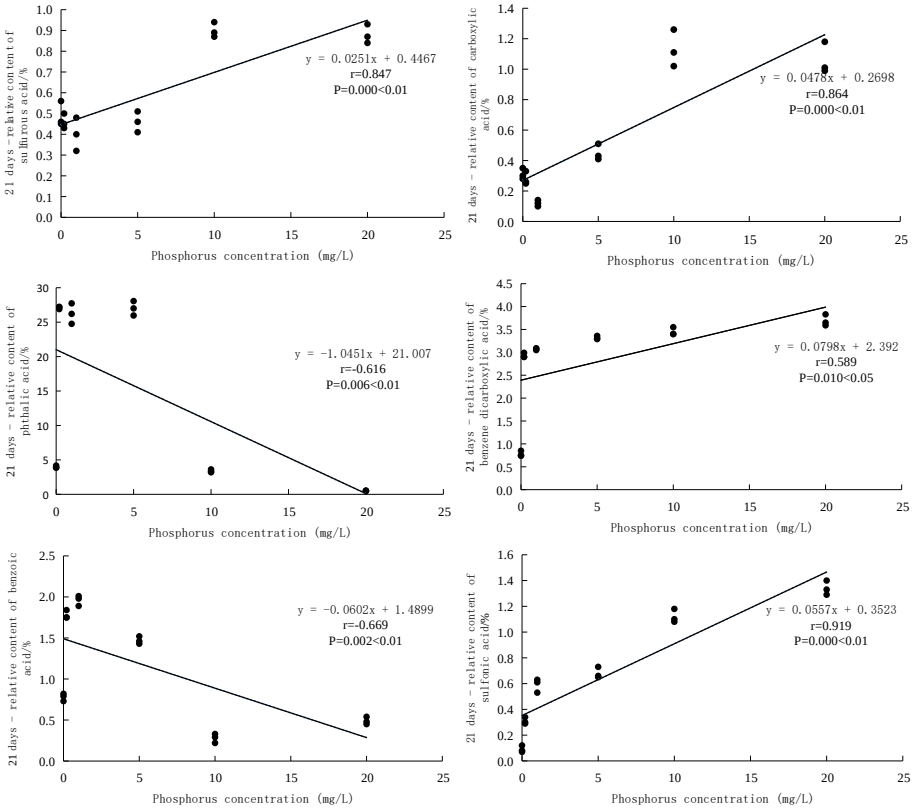

Fig. 4: Correlation analysis between phosphorus concentration and organic acid at 21 days of stress duration

and sulfonic acid contents and phosphorus stress concentration at 7 days of culture ($P > 0.05$). When the stress duration was 14 days (Fig. 3), there was a very significant positive correlation between the concentration of sulfite and phosphorus stress (r=0.865, P=0.000). There was a significant negative correlation between carbonic acid and phenyldicarboxylic acid and phosphorus stress concentration (r = -0.500, P = 0.035; r = -0.493, P = 0.037), that is, with the increase in phosphorus concentration, the exudation of carbonate and phenyldicarboxylic acid in root exudates decreased gradually after 14 days of culture. The relative contents of carboxylic acid, phthalic acid, oxalic

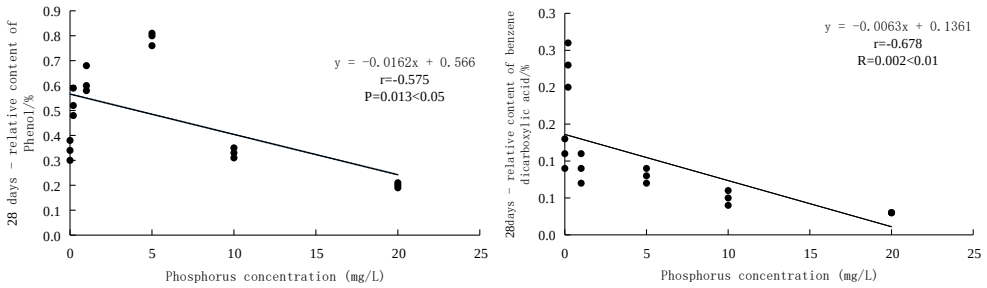

Fig. 5: Correlation analysis between phosphorus concentration and organic acid at 28 days of stress duration

acid, benzoic acid, sulfonic acid, and total organic acid had no significant correlation with phosphorus concentration after 14 days of culture ($P > 0.05$). When the stress duration was 21 days (Fig. 4), there was a very significant positive correlation between sulfurous acid, carboxylic acid and sulfonic acid concentration and phosphorus stress concentration (r = 0.847, P = 0.000; r = 0.864, P = 0.000; r = 0.919, P = 0.000). There was a significant positive correlation between benzene dicarbonic acid and phosphorus stress concentration (r = 0.589, P = 0.010), and very significant negative correlation between phthalic acid, benzoic acid and phosphorus stress concentration (r = -0.616, P = 0.006; r = -0.669, P = 0.002). There was no significant correlation between the amount of calcium carbonate, oxalic acid and total organic acid in phosphate stress culture for 21 days ($P > 0.05$). When the stress duration was 28 days (Fig. 5), there was a significant negative correlation between carbonation and phosphorus stress concentration (r = -0.575, P = 0.013). There was a significant negative correlation between benzene dicarbonic acid and phosphorus stress concentration (r = -0.678, P = 0.002), with the increase in phosphorus concentration, the amount of carbonate and benzene dicarbonic acid in root exudates at 28 days decreased gradually. Sulfurous acid, carboxylic acid, phthalic acid, oxalic acid, benzoic acid, sulfonic acid, and total organic acid had no significant correlation with phosphorus concentration at 28 days of phosphorus stress ($P > 0.05$).

Discussion

Root system is an important part of energy exchange between plants and the surrounding environment. Plants adjust root exudate types and contents to adapt to environmental changes and play a major role in regulating the supply of micronutrient elements in the rhizosphere. The diversity of root exudates is the result of adaptation of different species to their living media (Tian *et al.* 2000; Qin *et al.* 2011; Chen *et al.* 2017). Root exudates can change the environment of plant roots by adjusting the secretion of organic acids, activate insoluble phosphorus in nutriental elements, and increase the absorption of nutrients in plants (Duan 2003; Zhang 2009). In the wetland's ecosystem where eutrophication is becoming a serious concern, the study on the effects of phosphorus stress on root exudates of wetland plants can clarify the rhizosphere measures of wetland management.

With the increase in culture time and phosphorus stress concentration, the amount of organic acids in *H. verticillata* root exudates varied greatly at different concentration levels and culture periods. After 7 days of culture, organic acids in *H. verticillata* roots exudates, such as carbonic acid, phthalic acid, benzoic acid, and total organic acid, increased with the increase in phosphorus concentration. At 14 days, only sulfite secretion increased with phosphorus concentration, while at 21 days, the secretion of sulfurous acid, carboxylic acid, phenyldicarboxylic acid, and sulfonic acid increased with the increase in phosphorus concentration, and no increase was observed at day 28. The reason for this phenomenon may be that at 7 days, the plant was exposed to phosphorus stress environment, the root system was sensitive, and the rhizosphere environment was regulated by increasing the exudation of organic acid. *H. verticillata* is a wetland plant, with a fast growth rate with culture time. As *H. verticillata* slowly approaches the aging stage, where the root secretion capacity is weakened, it may lead to the reduction in acid secretion. The reasons for the decrease in specific secretions need to be further verified.

Total organic acids were secreted the most at 1 and 5 mg/L, and decreased significantly at 20 mg/L phosphorus concentration, indicating that the phosphorus concentration most suitable for the growth of *H. verticillata* may be in the 1–5 mg/L range. Phosphorus concentrations above or below that range may have been far less or more than the amount needed by the *H. verticillate*, respectively. High phosphorus concentrations can destroy plant root tissue and lead to a decline in plant root exudation. Phthalic acid is the main organic acid secreted by the root system of *H. verticillata* at all culture levels, which is different from the previous studies on plants such as *Brassica napus and soybean* in response to phosphorus control stress, wherein the main acid secreted is malic acid (Duan 2003; Zhang *et al.* 2011), while *Clove* secretes citric acid, malic acid, and succinic acid (Montague 1984). *Broussonetia papyrifera* and *Orychophragmus violaceus* primarily secrete oxalic acid, citric acid, and malic acid (Zhao and Wu 2014).

Plant diversity might explain the reason underlying the secretion of various acids. *H. verticillata* is a typical wetland submerged plant, different from the terrestrial plant growth environment. The concentrations of phosphorus stress were phosphorus deficiency, low concentration and high concentration of phosphorus, and the relative content of phthalic acid was the highest in the organic acids secreted under different culture time. This indicates that the high secretion of phthalic acid is the adaptation mechanism of diatoms in wetland submerged plants under the stress of external nutrient elements.

Conclusion

Under phosphorus stress for 7 days, the least amount of carbonic acid, phthalic acid, phenyldicarboxylic acid, benzoic acid, and total organic acid was observed. With the increase in phosphorus concentration, the secretion of benzoic acid and phthalic acid initially decreased followed by an increase. With the increase in phosphorus concentration, the secretion of benzoic acid and phthalic acid increased first and then decreased. Oxalic acid secretion gradually decreased with the increase in phosphorus concentration, and the amount of carbonate, phthalic acid, benzene dicarbonic acid, and benzoic acid gradually increased with the increase in phosphorus stress concentration. On days 14, 21 and 28, the least secretion of phthalic acid, benzene dicarbonic acid, and total organic acid was observed in the condition of no phosphorus stress, whereas sulfurous acid, carboxylic acid, sulfonic acid, and benzene dicarbonic acid secretion gradually increased with the increase in phosphorus stress concentration. The results indicate that the secretion of organic acids in the roots of *H. verticillata* is closely related to the concentration of phosphorus stress and the duration of stress period. Phosphorus stress causes the roots to adjust the secretion of organic acids actively. Phthalic acid is the main organic acid secreted by *H. verticillata* roots at all culture levels, indicating that the regulation of phthalic acid secretion by the root system under phosphorus stress is an important mechanism of active adaptation to environmental changes.

Acknowledgements

National Science Foundation of China (No. 31760149;31860235), Fund to Key Research and Development Program of Yunnan Provincial (No.2018BB018), Fund Project to The forestry science and technology innovation platform project of The State Forestry Administration (2019132161; 2019-YN-13).

References

Akhtar MS, Y Oki, Y Nakashima, M Nishigaki, T Adachi, T Kamigaki (2016). Microcosm investigation on differential potential of free floating azolla macrophytes for phytoremediation of P-controlled water eutrophication. *Intl J Agric Biol* 18:204–212

Chen BY (2009). Activation of phosphorus in Red soil by Root exudates of Wheat *Vicia faba* under phosphorus stress. *Yunnan Agric Univ* 24:869–875

Chen F, YJ Meng, HW Shuai, XF Luo, WG Zhou, JW Liu, WY Yang, K Shu (2017). Effects of plant allelochemicals on seed germination and its ecological significance. *Chin J Ecol Agric* 25:36–46

Deng Y, HL Guan, KJ Dai, WJ Zhou, YX Shen (2006). Effects of phosphorus supply on Morphogenesis and Organic Acid secretion in Rhizosphere of Pinus yunnanensis seedlings. *J. Yunnan Univ Nat Sci Edit* 28:358–363

Duan HY (2003). *Studies on Nutritional Physiology and Genetic Behavior of High Phosphorus Efficiency in Brassica napus* L. Central China Agriculture University, Wuhan, China

Hinsinger P (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil* 237:173–195

Horchani F, P Gallusci, P Baldet, C Cabasson, M Maucourt, D Rolin, S Aschi-Smiti, P Raymond (2008). Prolonged root hypoxia induces ammonium accumulation and decreases the nutritional quality of tomato fruits. *J Plant Physiol* 165:1352–1359

Hou XL, CG Yuan, XP Li, YC Ren, YM Luo, DK Wang (2018). Effects of nitrogen and phosphorus concentrations in Dianchi Lake on the interannual changes of Blue, Green and Diatom. *J Water Ecol* 39:16–22

Huang YF, QY Yang, TP Zhang, JT He (2014). Root exudation characteristics of two plants under hydroponics and their relationship with pollutant removal. *J Ecol* 33:373–379

Huiyong YU, G Shen, X Gao (2013). Determination of tobacco root exudates by GC-MS. *Acta Tabac. Sin* 19:64–72

Kuang YW, DZ Wen, CW Zhong, GY Zhou (2003). Root exudates and their role in phytoremediation. *J Plant Ecol* 27:709–717

Li DH, CL Xiang, YQ Jiang, LY He (2005). Difference of Organic Acid secretion in different Rice varieties under low phosphorus stress. *Chin Agric Sci Bull* 21:186–188

Li H, XR Yang, B Weng, JQ Su, S Nie (2016). Comparison of the removal of Total nitrogen and phosphorus from simulated eutrophication Water by four kinds of eutrophication plants, four submerged plants and their combined Communities. *Wetl Sci* 14:163–172

Liu TT, YT Qin, YY Wu, J Zhang, YR Zhang (2017). Solvent extraction of organic sulfur compounds from tiller onion and its GC-MS analysis. *Food Sci* 38:151–156

Lu SL, HY Hu, YX Sun, J Yang (2009). Study on growth and root exudates of three wetland plants under hydroponics. *Environ Sci* 30:1901–1905

Ma RC, Q Liu, H Li, XY Shi, J Li (2017). Effects of phosphorus deficiency stress on root development and nutrient uptake of sweet potato in early stage. *J North Chin Agric* 32:171–176

Montague BW (1984). *Emittance growth from multiple scattering in the plasma beat-wave accelerator*. CERN, Geneva, Switzerland

Qin BQ, G Gao, GW Zhu, YL Zhang, YZ Song, XM Tang, H Xu, JM Deng (2013). Lake eutrophication and its ecosystem response. *Sci Bull* 58:855–864

Qin L, H Jiang, J Tian, J Zhao, H Liao (2011). Rhizobia enhance acquisition of phosphorus from different sources by soybean plants. *Plant Soil* 349:25–36

Rellan-Alvarez R, S Andaluz, J Rodriguez-Celma, G Wohlgemut, G Zocchi, A Alvarez-Fernandez, O Fiehn, AF Lopez-Millan, J Abadia (2010). Changes in the proteomic and metabolic profiles of Beta vulgaris root tips in response to iron deficiency and resupply. *BMC Plant Biol* 10:120–135

Roy ED (2017). Phosphorus recovery and recycling with ecological engineering: A review. *Ecol Eng* 98:213–227

Tang X, G Gao, J Chao, X Wang, G Zhu, B Qin (2010). Dynamics of organic aggregate associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in China. *Limnol Oceanogr* 55:469–480

Tian ZM, FL Qin, B Wang (2003). Comparative study on collecting methods of Root exudates from Phosphorus-deficient White Lupin. *J NW Univ Agric For Sci-Technol* (*Nat Sci Edit*) 31:154–158

Tian ZM, CJ Li, CW, ZJ Zhao (2000). Comparison of Organic acids secreted from Root Tips of Phosphorus-deficient White Lupin Bean. *Mol Plants* 26:317–322

Wang RL, JY Liu, TR Guo (2014). Effects of aluminum toxicity and low phosphorus stress on organic acid secretion from rice seedling growth roots. *Anhui Agric Sci* 6:1603–1606

Wei XJ, M Liu, L Zheng, AZ Ding, Y Li, X Zhao, MX Liu (2016). Effect of salt stress on root exudates of Reed. *J Beij Normal Univ* (*Nat Sci Edit*) 52:44–48

You HL, XL Gang, JH Jiang, JX Xu, JM Deng, XL Wang (2013). Research progress of growth dynamics and environmental adaptability of roots of wetland plants. *Resour Environ Yangtze Basin* 1:52–58

Yu Y, M Zhang, SQ Qian, DM Li, FX Kong (2010). Present situation and Evolution of Lake Water quality in Yungui Plateau. *Lake Sci* 22:820–828

Zhang HW (2009). *Physiological Mechanism of phosphorus efficiency in Brassica napus* L. Central China Agriculture University

Zhang RM, D Zhang, HW Chen, J Bai, Y Gao (2007). Study on extraction method of root exudates from *Haloxylon ammodendron* seedling. *Resour Environ Arid Areas* 21:153–157

Zhang ZH, Y Chen, SF Han, MC Zhang, DM Wang (2011). Effects of low phosphorus stress on root growth characteristics, secretion and organic acids in soybean. *Chin J Oil Crops* 33:135–140

Zhang ZR, XF Zhang, J Guo, JQ Wang (2018a). Experimental study on purification of eutrophication water by three new floating bed plants. *J Univ Sci-Technol Chin* 3:221–228

Zhang ZX, JK Liu, ZM Zhang, MX Zhang (2018b). Comparison of removal efficiency of nitrogen and phosphorus in water by artificial floating Island planted with different plants and their combinations. *Wetl Sci* 16:273–278

Zhao HC, SR Wang, XC Jin, QY Bu, JH Liu (2008). Utilization and transformation of phosphorus forms in sediments and soils by *Hydrilla Verticillata*. *Lake Sci* 20:315–322

Zhao K, Y Wu (2014). Rhizosphere calcareous soil P-extraction at the expense of organic carbon from root-exuded organic acids induced by phosphorus deficiency in several plant species. *Soil Sci Plant Nutr* 60:640–650

Zhao K, B Zhou, MA Wanzheng (2016). The influence of different environmental stresses on root-exuded organic acids: A review. *Soils* 48:235–240

Zhou JC, XC Wang, YH Deng, XK Lin, Y Wang (2016). Study on temporal and Spatial variation of water quality and water quality zonation of wetland lakes in the Caohai Plateau. *J Water Ecol* 37:24–30

Zhou JC, XC Wang, YH Deng, XK Lin, Y Wang (2011). Effects of phosphorus stress on the root morphology and root exudates in different sugar beet genotypes. *Chin Agric Sci Bull* 11:1151–1154

Zou YH, RY Zhang, JA Chen, LY Wang, DP Lu (2018). Application of clay minerals in the control of phosphorus pollution in eutrophic water and sediment. *Adv Earth Sci* 33:578–589