

## **Vicia Villosa Root Exudates Improve the Mobilization of Available P, K, Zn and Fe in Three Types of Purple Soil**

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**Keywords:** Hairy vetch, purple soil, root exudates, available nutrients

**Abstract.** Root exudates play a major role in the mobilization of sparingly soluble nutrients in the rhizosphere. Purple soils have poor fertility as a result of soil erosion and certain soil characteristics. In this study, we examined how root exudates of hairy vetch (*Vicia villosa*) affected soil-available nutrients in three types of purple soil, by using solution-cultivation and soil-cultivation methods. Root exudates were collected by solution cultivation and a soil-cultivation experiment was carried out with two exudate treatments (low amount and high amount). In all three types of purple soil, high amount of root exudates significantly increased available P, K, Zn, Fe ( $P < 0.05$ ). The activation effects of exudates on soil available P and Zn were most substantial in alkaline purple soils. Soil available K was most primary in neutral purple soils, and soil available Fe was major in acidic purple soils. These results suggest that planting hairy vetch in winter fallow increase specific nutrients of purple soils in the study area because of the mobilization of root exudates and different types of soil.

### **Introduction**

Purple soil is a type of non-zonal soil that develops by the weathering of purple sandstone (composed of the ferric oxide and manganese oxide that make it purple) and purple shale rock under subtropical and tropical monsoon climates. As a result of the severe soil erosion and serious land degradation predominant in the purple soil region, 48.8% of the area covered by purple soil is damaged by soil erosion [1, 2]. Crop production, is deteriorating as a result of cultivated purple soils becoming compacted, hardened, shallow [3], lower in moisture and reduced in fertility. Improving the quality and fertility of purple soils has therefore become an urgent need to ensure their sustainability.

Root exudates are substances that are released from plant roots into the rhizosphere. Root exudates consist of organic compounds of both low and high molecular weights [4, 5]. Root exudates are an important medium with which plants exchange material, energy and information with the soil, and thus are an organic source and sink of rhizosphere micro-ecosystems. Previous studies have shown that root exudates of common bean can activate Al-P and Fe-P [6]. Root exudates of grain amaranth positively affect available K [7]. Release of phytosiderophores in the graminaceous species mobilizes zinc and iron with zinc deficiency [8, 9].

Hairy vetch (*Vicia villosa* Roth) is widely used in agroecosystems as a legume cover crop and green manure [10, 11]. It is native to Europe and western Asia [12], and is distributed mainly in the Sichuan, Jiangsu, Anhui and Henan provinces of China. Current research on hairy vetch is focused on its use as a green manure to improve soil fertility [13], how multi-cropping hairy vetch after wheat can have a yield-increasing effect [14], and how hairy vetch root exudates can have a suppression effect on weeds and pests [15,16]. Few studies have investigated the effect of hairy vetch root exudates on soil fertility. The purpose of our study was to examine how hairy vetch root exudates affect soil fertility in three types of purple soil using the solution-cultivation and soil-cultivation methods.

## Materials and Methods

### Experimental Materials and Soil

Hairy vetch seedlings were purchased from Henan HuaFeng Prataculture Technology Co., Ltd (Zhongzhou, Henan Province, China). Soil samples consisted of three types of purple soils (acidic, alkaline and neutral). Acidic soil was collected from Jiangjin, Chongqing, China. Alkaline soil was collected from Tongnan, Chongqing, China. Neutral soil was collected from Hechuan, Chongqing, China. Samples were collected from the plough layer (0–20 cm) of each soil. We selected five sites for each soil type on the basis of soil pH, soil organic matter, alkali-hydrolysable N content, available P content, available K content, available Fe content, available Zn content, Total N content, Total P content and Total K content. A summary of soil properties is shown in Table 1. Directly after collection from the field, any discernable roots was removed and the samples were air-dried, ground and sieved [17]. This study was conducted in a laboratory cultivation room, Department of Plant Nutrition at Southwest University, from March to June 2014.

**Table 1** Characteristics of the three types of purple soils used in the experiment. (mean±SD, N=8)

Type of purple soil	Available N (mg·kg <sup>-1</sup> )	Available P (mg·kg <sup>-1</sup> )	Available K (mg·kg <sup>-1</sup> )	Available Fe (mg·kg <sup>-1</sup> )	Available Zn (mg·kg <sup>-1</sup> )	Total N (g·kg <sup>-1</sup> )	Total P (g·kg <sup>-1</sup> )	Total K (g·kg <sup>-1</sup> )	Organic matter (g·kg <sup>-1</sup> )	pH
Acidic	62.52±1.78	11.71±1.23	14.52±0.46	2.35±0.21	1.00±0.02	0.64±0.03	0.47±0.01	11.80±1.56	15.11±1.56	5.50±0.15
Alkaline	53.63±2.72	3.35±0.35	20.09±1.34	0.71±0.07	0.69±0.01	0.85±0.05	0.77±0.02	21.12±2.34	13.76±1.23	8.64±0.23
Neutral	71.08±1.98	11.19±2.01	50.23±2.46	2.22±0.16	1.41±0.08	0.88±0.04	0.85±0.04	15.31±2.19	10.28±0.98	7.44±0.11

### Solution Culture Experiment

Hairy vetch seeds of the same size were selected using their dimensions and surface-sterilized in 15% hydrogen peroxide for 15 min, then rinsed several times in deionized water. Seeds were evenly scattered in a tray containing wet quartz sand and covered with moist filter paper. Trays were placed in a dark inoculation room for germination at 25°C. After germination, hairy vetch seedlings were cultured with hydroponically in growth chambers (day/night cycle: 16 h at 23°C/8 h at 19°C). The cultivation system was bubbled with air to supply sufficient O<sub>2</sub> and to circulate nutrients. When seedlings reached the third-leaf stage they were gently removed from the tray and the roots were washed several times with deionized water. Every 15 seedlings were transferred into a 2 L pot filled with half-strength nutrient solution (diluted with distilled water), grown for one week and then transferred into the same-size pot containing whole nutrient solution for culturing. We planted 10 pots for collecting root exudates of hairy vetch. Root exudates were collected from pots on 40 d after transplanting. At that time, Hairy vetch was in a vigorous period. The specific collection method was as follows [18]: 2 h into the light treatment on day 40, 15 plants were gently taken out of each pot and the roots were washed four times with deionized water. Roots were transferred to 1 L beakers containing 450 mL of deionized water and covered with aluminum foil [19]. After 4 h, the solutions from all plants were collected and aggregated. To concentrate the exudates, the solutions were processed 40 times in a vacuum rotary evaporator. The concentrated solution of the root exudates of 150 plants were filtered by 0.45 μm Millipore membranes and stored at -20°C until use. Each 0.75 mL concentrated solution was equivalent to the amount of root exudates secreted by one plant for 4 h.

### Soil-Cultivation Experiment

The soil-cultivation experiment was carried out using the method of Zhao et al [18]. There were nine treatment combinations in a full-factorial setup. The three purple soil types were acidic, alkaline and neutral. The three root exudate treatments were high concentration (12 mL root exudates above, as follows), low concentration (6 mL root exudates plus 6 mL deionized water),

and a control (12 mL deionized water). We added 60 g of air-dried and sieved (2 mm) purple soil to each 100 mL beaker. The exudate solutions were then added slowly and mixed. The water-filled pore space (WFPS) was 80%. Beakers were kept in a dark, constant-temperature box at 25°C for 15 d. Each treatment was repeated four times. Beakers were weighed daily and neutral deionized water was added to replace evaporated water. After 15 d, soil samples were removed from beakers and all samples were prepared according to the standard method of determination index [18].

### Determination Index and Method

The content of alkali-hydrolysable N, available P, available K, available Zn and Fe in soil was measured by the methods from Li et al. [20].

### Statistical Analysis

All data were analyzed with SPSS 18.0 and Microsoft Excel 2007. Differences between treatment means were tested by one-way analysis of variance (ANOVA), and if treatment effects were found to be significant at the level of  $P < 0.05$  [21].

## Results and Discussion

### Effect of Hairy Vetch Root Exudates on Available P Contents of Purple Soil

The content of available P increased significantly in acidic and alkali soils after adding concentrated solutions of exudates into the soil. Moreover, available P increased with the increase of the amount of root exudates. It showed that, compared with the control, the available P increased by 1.49 times and 1.36 times after adding concentrated solutions of exudates in acidic soils, and 1.40 times and 1.08 times in neutral soils, while 4.64 times and 3.41 times of the control respectively in alkali soils. Consequently, the increasing effect of exudates on available P was most substantial in alkali soils. It may be related to higher content of insoluble P compounds (such as Ca-P) in alkaline purple soil [22-24], although the specific reason remains unknown.

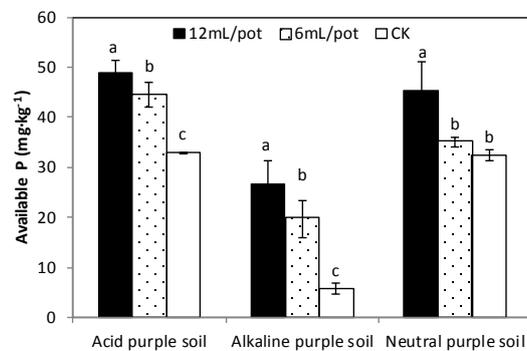


Fig. 1 Effect of hairy vetch root exudates on available P contents of purple soil

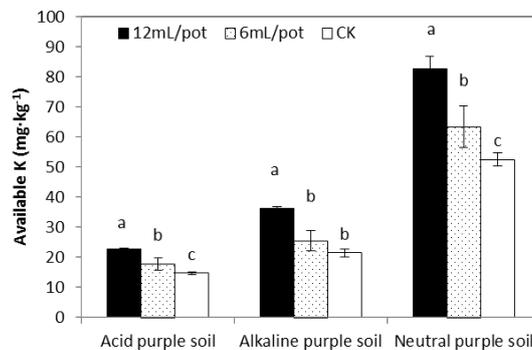


Fig. 2 Effect of hairy vetch root exudates on available K contents of purple soil

Note: Data are the average of four replicates. Different letters indicated significant difference

among different treatments with the same type of soil at  $P < 0.05$ . Root exudates of hairy vetch was used in the experiment, namely every 0.75 mL concentrated hairy vetch root exudates was equivalent to the exudate secreted by one plant for 4 hours. The same is for figures below.

### Effect of Hairy Vetch Root Exudates on Available K Contents of Purple Soil

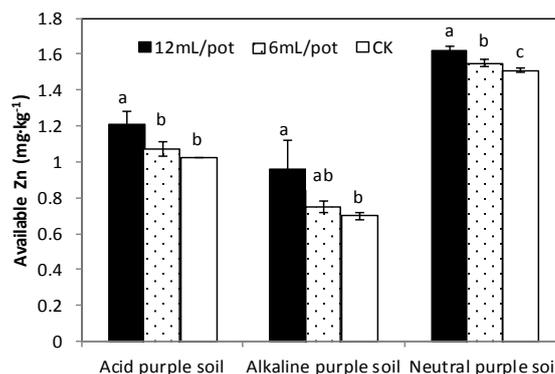
The content of available K increased significantly ( $P = 0.001$ ) in acidic and neutral soils after adding concentrated solutions of exudates into the soil (Fig.2). Moreover, available K increases with the increase of the amount of root exudates in both types of soils. Compared with the control, the content of available K increased significantly in alkali soils only after adding high amount of exudates into the soil ( $P = 0.00$ ). It showed that, compared with the control, after adding high and low amount of concentrated solutions of exudates, the available k increased by 8.04 and 3.09  $\text{mg.kg}^{-1}$  in acidic soils, and 14.75 and 4.04  $\text{mg.kg}^{-1}$  in alkali soils. However, the available k increased by 30.16 and 10.92  $\text{mg.kg}^{-1}$  respectively in neutral soils (Fig.2). Therefore, the increase in available K was most substantial in neutral soils treated with exudates. It may be related to easily form metal-organic complexes with the metal ions in the mineral structure in neutral soils, which can accelerate the decomposition of minerals [25, 26].

### Effect of Hairy Vetch Root Exudates on Available Fe Contents of Purple Soil

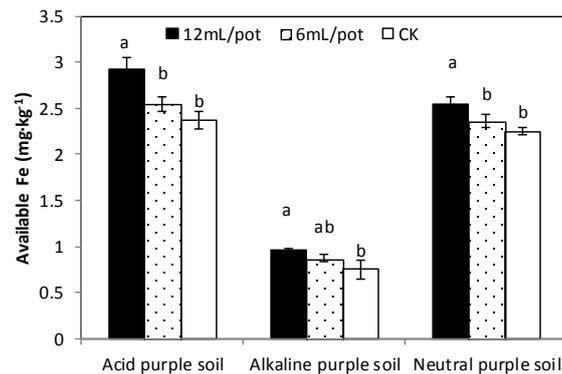
Compared with the control, the content of available Fe increased significantly in three types of soil only after adding high amount of exudates into the soil ( $p_{acid}=0.002$ ,  $p_{alkali}=0.020$ ,  $p_{neutral}=0.003$ ). Compared with the control, the available Fe was increased by 0.55  $\text{mg.kg}^{-1}$  after adding high amount of exudates in acidic soils, while increased by 0.21  $\text{mg.kg}^{-1}$  in alkali soils, and increased by 0.3  $\text{mg.kg}^{-1}$  in neutral soils (Fig.3). Thus, the increasing effect of exudates on available Fe was most substantial in acidic soils. Fe mobility in acidic soils is enhanced by the presence of organic acids from root exudates, which can further direct formation of Fe complexes [27], and lower pH in acidic soils means it is easy to be replaced by  $\text{H}^+$  for  $\text{Fe}^{2+}$  on the surface of the soil [28].

### Effect of Hairy Vetch Root Exudates on Available Zn Contents of Purple Soil

Compared to the control, the content of available Zn increased significantly in acidic ( $P=0.002$ ) and alkali ( $P=0.013$ ) soils only after adding high amount of exudates into the soil. The content of available Zn increased significantly in neutral soils after adding 12 mL or 6 mL concentrated solutions of exudates into the soil compared with the control ( $P=0.033$ ). Moreover, available Zn increases with the increase of the amount of root exudates in neutral soils. Compared with the control, the available Zn was increased by 0.19  $\text{mg.kg}^{-1}$  after adding high amount of exudates in acidic soils, while by 0.26  $\text{mg.kg}^{-1}$  in alkali soils, and by 0.11  $\text{mg.kg}^{-1}$  in neutral soils. Consequently, the increasing effect of exudates on available Zn was most substantial in alkali soils. Root exudates reduce the concentration of  $\text{Ca}^{2+}$  in alkali soils directly by forming a more stable complexes with  $\text{Ca}^{2+}$ , thus reducing the adsorption and fixation of Zn by calcium compounds so that  $\text{Zn}^{2+}$  is released into the soil. Further studies are needed to investigate how exudates affect available Zn in alkali soils.



**Fig. 3** Effect of hairy vetch root exudates on available Fe contents of purple soil



**Fig. 4** Effect of hairy vetch root exudates on available Zn contents of purple soil

## Conclusions

In this study, addition of hairy vetch root exudates resulted in a significant increase in available P, K, Zn, and Fe. The treatment with a higher concentration of root exudates showed the most significant positive effect on soil-available nutrients. However, the effect was different in purple soils of different types. Our results suggest that planting hairy vetch can activate P, K, Zn, and Fe in purple soils. It showed that the activation effects of exudates on soil available P and Zn were most substantial in alkaline purple soils. Soil available K was most primary in neutral purple soils, and soil available Fe in acidic purple soil.

## Acknowledgments

This study was supported by the National Key Research and Development Program of China (2017YFD0200100), the National Green Manure Industry Technology System Foundation of China (CARS-22), the National Natural Science Foundation of China (40801109).

## References

- [1] Lin, C.W., Tu, S.H., Huang, J.J., Chen, Y.B., The effects of plant hedgerows on soil erosion and soil fertility on sloping farmland in the purple soil area. *Acta Ecol. Sin.* 27(2007) 2191-2198.
- [2] Tang J. L., Zhu B., Wang T., Cheng X.Q., Gao M.R., Lin H., Subsurface Flow Processes in Sloping Cropland of Purple Soil. *J. Mt. Sci.* 9(2012) 1-9.
- [3] Zhu, B., Chen, S., You, X., Peng, K., Zhang, X., Soil fertility restoration on degraded upland of purple soil. *Acta Pedologica Sini.* 39 (2002) 743-749.
- [4] Bertin, C., Yang, X.H., Weston, L. A., The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil* 256 (2003) 67-83.
- [5] Dijkstra, F. A., Cheng, W.X., Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecol. Lett.* 10(2007) 1046-1053.
- [6] Shen, H., Yan, X. L., Zhao, M., Zheng, S.L., Wang, X.R., Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. *Environ. Exp. Bot.* 48 (2002) 1-9.
- [7] Li, T., Ma, G., Zhang, X., Root exudates of potassium-enrichment genotype grain amaranth and their activation on soil mineral potassium. *Chin. J. Appl. Ecol.* 17 (2006)368.
- [8] Zhang F.S., Romheld, V., Marschner, H., Release of zinc mobilizing root exudates in different

species as affected by zinc nutritional status. *J. Plant Nutr.* 14(1991) 675-686.

- [9] Hopkins B. G., Whitney D. A., Lamond R. E., Jolley V. D., Phytosiderophore released by sorghum, wheat and corn under zinc deficiency. *J. Plant Nutr.* 21(1998)2623-2637.
- [10] Utomo, M., Frye, W. W., Blevins, R. L., Sustaining soil nitrogen for corn using hairy vetch cover crop. *Agronomy J.* 82(1990) 979-983.
- [11] Power, J. F., Doran, J. W., Koerner, P. T., Hairy vetch as a winter cover crop for dryland corn production. *J. Prod. Agric.*, 4 (1991) 62-67.
- [12] Mothapo, N. V., Grossman, J. M., Sooksa-Nguan, T., Maul, J., Brauer, S. L., Shi, W., Cropping history affects nodulation and symbiotic efficiency of distinct hairy vetch genotypes with resident soil rhizobia. *Biol. Fert. Soils*, 49 (2013) 871-879.
- [13] Jongho, S., Hojin, L., Ilbong, H., Siju, K., Chungkuk, K., Hyeonsuk, J. Use of hairy vetch green manure as nitrogen fertilizer for corn production. *Korean J. Crop Sci.* 45 (2000) 294-299.
- [14] Duan, Y., Cao, W.D, Tuo, D.B., Zhang, J., Yao, J.Q., An, H., Study on the yield-increasing effect of multi-cropping vicia villosa after wheat. *Inner Mon. Agr. Sci. Tec.* 5(2010)42-43.
- [15] Abig-Ghanem, R., Carpenter-Boggs, L., Smith, J. L., Cultivar effects on nitrogen fixation in peas and lentils. *Biol. Fert. Soils* 47(2011) 115-120.
- [16] Campiglia, E., Caporali, F., Radicetti, E., Mancinelli, R., Hairy vetch (*Vicia villosa* Roth.) cover crop residue management for improving weed control and yield in no-tillage tomato (*Lycopersicon esculentum* Mill.) production. *Europ. J. Agronomy* 33 (2010) 94-102.
- [17] Nebiyu, A., Diels, J., Boeckx, P., Phosphorus use efficiency of improved faba bean (*Vicia faba*) varieties in low-input agro-ecosystems. *J. Plant Nutr. Soil Sci.* 179(2016) 347-354.
- [18] Bowsher, A. W., Ali, R., Harding, S. A., Tsai, C.J., Donovan, L. A., Analysis of wild sunflower root exudates using gas chromatography-mass spectrometry. *J. Plant Nutr. Soil Sci.* 178(2015) 776-786.
- [19] Zhao, J.L., Cheng, C.Q., G. X.Y., Liu, B., Effects of root exudates from *Mirabilis Jalapa* on soil microenvironment. *J. Henan Nor. Univ.* 42(2014) 95-99.
- [20] Li, X.P., Mu, Y.H., Cheng, Y.B., Liu, X.G., Hai, N., Effects of intercropping sugarcane and soybean on growth, rhizosphere soil microbes, nitrogen and phosphorus availability. *Acta Physiol. Plant.* 35 (2013) 1113-1119.
- [21] Nardi, P., Akutsu, M., Pariasca-Tanaka, J., Wissuwa, M., Effect of methyl 3-4-hydroxyphenyl propionate, a sorghum, root exudate, on n dynamic, potential nitrification activity and abundance of ammonia-oxidizing bacteria and archaea. *Plant Soil* 367 (2013) 627-637.
- [22] Jones, D. L., Darrah, P. R., Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* 166(1994) 247-257.
- [23] Ryan, P. R., Delhaize, E., Jones, D.L., Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol. Mol. Biol.* 52 (2001) 527-560.
- [24] Gerke, J., The acquisition of phosphate by higher plants: effect of carboxylate release by the roots-a critical review. *J. Plant Nutr. Soil Sci.* 178 (2015) 351-364.
- [25] Chen, Y. L., Guo, Y. Q., Han, S. J., Zou, C. J., Zhou, Y. M., Cheng, G. L., Effect of root derived organic acids on the activation of nutrients in the rhizosphere soil. *J. Forest Res.* 13 (2002): 115-118.
- [26] Najafi-Ghiri, M. and Jaber, H. R., Effect of soil minerals on potassium release from soil fractions by different extractants. *Arid Land Res. Manag.* 27 (2013) 111-127.

- [27] Carvalhais, L. C., Dennis, P. G., Fedoseyenko, D., Hajirezaei, M. R., Borriss, R., vonS Wiren, N., Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *J. Plant Nutr. Soil Sci.* 174 (2011) 3-11.
- [28] Jones, D. L., Organic acids in the rhizosphere – a critical review. *Plant Soil* 205 (1998) 25-44.