

Introduction

There are 56.9 million ha of red acidic soils in China and most of them have low bioavailable phosphorus (P). Phosphorus is one of the key limiting factors for crop productions on these soils. Yunnan province at Southeastern China has 11.4 million ha of red soils, which accounts for 32% of the total farming area (Wang, 1996). The total P concentrations in these soils are approximately 0.9 g kg^{-1} , of which 26% is in the form of iron-phosphate (Fe-P) complex. The complex of Fe-P in red soils is not available or much less available to crops because of its low solubility. Roots of some monocot graminaceous species can exudate phytosiderophores under iron stress condition in acidic soils. 2'-deoxymugineic acid (DMA) was first isolated and identified in the root exudates of Fe deficient wheat (Nomoto et al., 1981; Ma et al. 2003), nine analogs have been isolated and identified from graminaceous species and cultivars (Ma et al. 2003; Ma, 2005). Phytosiderophores chelate iron and form iron phytosiderophores. The chelating constant of iron phytosiderophores is about 7.7×10^{33} and much greater than the solubility of Fe-P (7.7×10^{21}) (Wu et al., 2001). Therefore, phytosiderophores can replace P in the Fe-P complex and make P available to plants after Fe-P complex is converted into iron phytosiderophores.

Soybean and maize intercropping is a traditional and typical farming system, which has maintained crop productivity in China for many centuries and doubled crop productivity in the past 40 years (China Statistical Bureau, 2004). Nie et al. (2004) has shown that wheat root exudates (REs) significantly promoted P uptakes by intercropping wheat and bean. The objective of this study was to investigate the effects of REs from wheat on P uptake by soybean in the maize and soybean intercropping system.

Materials and methods

Production of wheat root exudates

The wheat (var. Yun K5) seeds were sown in quartz sand in a tray moistened with saturated CaSO_4 solution and placed at 25°C in the dark. At about 5 days after germination and when the first leaf extends to 2/3rd of the full size, two hundred seedlings were transferred into a pot (28 cm in diameter and 15 cm in height) containing nutrient solution and continuously aerated. The full strength nutrient solution without Fe had the following nutrients in molarity: K_2SO_4 0.75×10^{-3} , MgSO_4 0.65×10^{-3} , KCl 0.1×10^{-3} , $\text{Ca}(\text{NO}_3)_2$ 2.0×10^{-3} , KH_2PO_4 0.25×10^{-3} , H_3BO_3 1×10^{-6} , MnSO_4 1×10^{-6} , CuSO_4 1×10^{-7} , ZnSO_4 1×10^{-6} and $(\text{NH}_4)_6\text{Mo}_2\text{O}_4$ 5×10^{-9} . During the first 48 h, 1/3 strength of the nutrient solution was used and then replaced with full strength solution. The nutrient solution was changed every 3 days from day 5. The collection of REs was initiated when the seedlings showed slight iron deficiency symptoms. The detailed procedure of REs collection was described by Zheng et al. (2005). Briefly, the seedlings were placed into a shallow pan with 1 L deionized water for 4 hrs every morning for 10 days. The collected REs were stored in a freezer at -18°C . The REs extraction procedures were repeated 12 times and the total volume of REs extractions was about 450 L. Using a similar extraction procedure, Ma et al. (1994) collected wheat REs and identified DMA in REs.

Pot experiment

The experiment was conducted in a randomized block design with four treatments and four replicates. The four treatments were: a) maize and soybean intercropping with REs, b) maize and soybean intercropping without REs, c) soybean monoculture with REs, and d) soybean monoculture without REs.

Pots (30 cm in diameter and 25 cm in height) were filled with clay-loam soils (Ultisols) from a farmland in the central Yunnan province. Soil characteristics were: pH 6.4, organic matter 29.7 g kg⁻¹, total N 1.56 g kg⁻¹, total P 1.82 g kg⁻¹, total K 6.27 g kg⁻¹ and CaCO₃ 89.5 g kg⁻¹, available N 107.8 g kg⁻¹, Olsen P 18.34 g kg⁻¹, available K 112.7 mg kg⁻¹. Each pot received fertilizers as follows: 150 mg N kg⁻¹ as Ca(NO₃)₂·4H₂O, 100 mg K kg⁻¹ as KCl, 50 mg Mg kg⁻¹ as MgSO₄, 5 mg Zn kg⁻¹ as ZnSO₄·7H₂O, and 5 mg Cu kg⁻¹ as CuSO₄·5H₂O.

Before sowing, both corn (var. Yunyou 196) and soybean (var. 661) seeds were soaked first in a solution containing 500 ml water and 1-2 drops of 30% H₂O₂ for 15 min, second in a 500 ml saturated CaSO₄ solution for 30 min, and washed three times with deionized water. Two corn seeds and six soybean seeds were sown in the same pot for intercropping treatments and twelve soybean seeds were sown in each pot for monoculture treatments. After germination, seedlings were thinned to one for corn and three for soybean per pot for the intercropping treatment, and six soybean seedlings for the monoculture treatment. Nitrogen fertilizer was applied as urea at the rate of 3 g per pot at fourth leaf, milking, and filling stages of maize. Five hundred ml of the REs solution was applied to each of the pots labeled for REs treatments every 4 days. Five hundred ml of deionized water was applied to other pots for treatments without REs.

Sampling and analysis

Plants were harvested at 30, 52, 96, 107 and 120 days after sowing. The stems and leaves of the soybean were combined for each pot and dry matter and shoot P content was determined by standard procedures (Bao, 2002). The soil attached to the root was collected as rhizosphere soil. Olsen P in the rhizosphere soil was determined after extraction with 0.5M NaHCO₃ solution at pH 8.5 (Bao, 2002). The Al-P fraction in rhizosphere soil was determined after sequential extraction with 1 M NH₄Cl and 0.5 M NH₄F. The Fe-P fraction was extracted with 0.1 M NaOH (Bao, 2002).

Statistical analysis

The data was analyzed using the analysis of variance (ANOVA) in SAS (SAS Institute, 2009). The Duncan's Multiple Range Tests were used to compare the difference between two means (Hubbard, 2001).

Results and Discussion

The effects of REs application on Olsen P concentration of rhizosphere soil is shown in Fig. 1. The Olsen P concentrations of rhizosphere soil had a decreasing trend during the first 107 days, decreases being significant at 96 and 107 days. However, the Olsen P concentrations were increased at 120 days. It matched well with shoot dry matter and P contents in opposite way (Table 1 and Fig. 2). Olsen P was low when shoot matter and P were high during the stage of rapid plant growth because plants extracted more P from soil.

The Olsen P concentration with REs application was significantly lower than that without REs at 30, 52 and 96 days under monocultured system. The RE-induced decrease was 40.2% at 96 days. At 107 and 120 days, the Olsen P concentrations with REs application showed no significant differences compared to that without REs. Similarly the Olsen P concentration for intercropping treatments were unaffected by REs. The results did not fit the hypothesis well and may be caused by several reasons. First, water soluble P in soil was probably leached by irrigation water. Plants were irrigated every 4 days. Plants REs beaked Fe-P complex, formed iron phytosiderphores and released water soluble P (HPO_4^{2-} and H_2PO_4^-). These orthophosphates were readily leachable. In contrast, soils without REs had much less water soluble P to loss. Leaching loss was not significant for intercropping system probably because evapotranspiration (ET) rate in intercropping system was much greater than that in monocultured system. However, further study is needed to confirm the effects of soil moisture and ET on P loss. Second, Olsen extraction with 0.5N NaHCO_3 may no capable to extract all plant available P from acidic soils. Therefore, P fractionation was included in this experiment to provide better understanding for soil P.

Under monocultured, the Fe-P concentrations with REs showed much lower levels than that without REs (Table 2). It indicated that REs could chelate Fe and release P from Fe-P complex. Ishikawa et al. (2002) reported that pigeon pea REs solubilized iron bounded P. The Al-P concentrations in rhizosphere soil were not significantly affected by REs application of the intercropped soybean at harvest. REs used in this experiment was not strong enough to solubilize Al bounded P.

Phosphorus content in shoots of intercropped soybean with and without REs application reached the peak values of 204 and 76.9 mg per pot at 107 days, respectively, and subsequently dropped down to 37.7 and 22.6 mg per pot at 120 days (Fig.2). The decrease of shoot P contents may be attributed to low plant available P (Fig. 1) and the older age of leaves collected. Shoot P content of intercropped soybean with REs was significantly greater than that without REs in the last 13 d of the growth period while shoot P from REs treatment was 165.3% greater than that without REs at 107 days. Therefore, REs application improved the P uptake in the intercropped soybean. Similar results were reported for wheat and broadbean intercropping system (Nie et al., 2004). In that study, REs of wheat mobilized P-Fe in red soil and facilitated P uptake of intercropped and monocultured broadbean. The wheat/broadbean planting system showed better effects in red soils for mobilizing P-Fe than that for maize/soybean system (Zheng, et al. 2004). In the monocultured systems, the peak value of shoot P occurred also at 107 days. The value was 157.6 and 153.6 mg per pot for the treatments with and without REs, respectively. They were not significantly different at the harvest. As explained before, leaching loss of water soluble P may be a reason for low plant available P in soil and relatively low shoot P.

The results showed no significant difference between the treatments with and without REs in the early growth stages (30 and 52 days) for the shoot dry matter of the intercropped soybean (Table 1). The shoot dry matter of intercropped soybean from the treatments with REs was slightly greater at 96 days and 52.3% greater at 120 days than those from treatments without REs. This indicated that REs application significantly increased soybean growth in the intercropped system. Similar results have been reported before (Siqueria, 1991; Dakora, 2002).

Monocultured soybean with REs application had significantly greater shoot dry matter than intercropped soybean with REs in the early stages (30 and 52 days). At harvest (120 days), the

shoot dry matter of intercropped soybean without REs was significantly lower than that of monocultured soybean at 107 and 120 days, respectively. However, the shoot dry matter of intercropped soybean with REs was 76% greater than that of monocultured soybean.

Acknowledgements

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Table 1: Effects of root exudates (Res) application on shoot dry matter of soybean.

Treatment	Days after planting (d) (D.W. g/pot)				
	30	52	96	107	120
ICRE	2.6b	19.0b	73.7ab	88.3b	91.1b
IC	2.4b	18.0b	71.2b	80.1b	59.8c
MCRE	4.1a	28.6a	108.4a	123.6a	104.9a
MC	4.2a	31.8a	105.8a	147.3a	126.6a

Means with the different letters within columns are significantly different ($P < 0.05$).

ICRE - maize and soybean intercropping with REs; IC-maize and soybean intercropping without REs; MCRE-soybean monoculture with REs; and MC-soybean monoculture without REs.

Table 2: Effects of root exudates (REs) application on inorganic P fractions of rhizosphere soil.

Treatment	Al-P (mg/kg)	Fe-P (mg/kg)	Total-P (g/kg)	Fe-P% of TP
ICRE	22.43	295.39a	2.26	13.29
IC	26.94	308.79a	1.97	15.66
MCRE	17.35	258.46b	1.95	13.35
MC	20.72	289.60a	1.94	14.96

Means with the different letters within columns are significantly different ($P < 0.05$).

ICRE - maize and soybean intercropping with REs; IC-maize and soybean intercropping without REs; MCRE-soybean monoculture with REs; and MC-soybean monoculture without REs.

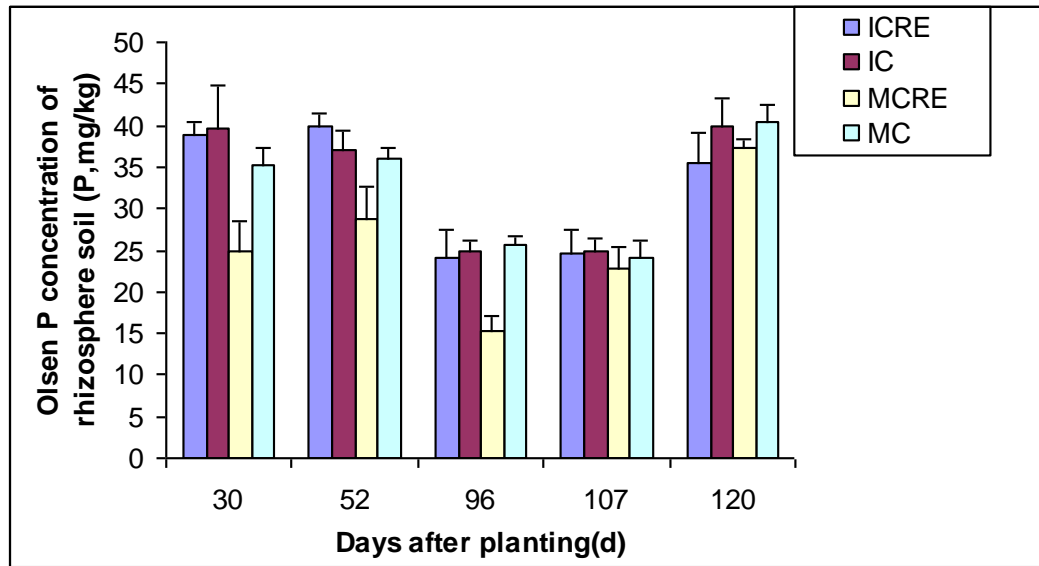


Figure 1: Effects of root exudates (REs) application on Olsen P concentration of rhizosphere soil in pot experiment. ICRE - maize and soybean intercropping with REs; IC-maize and soybean intercropping without REs; MCRE-soybean monoculture with REs; and MC-soybean monoculture without REs. Mean values are followed by SD.

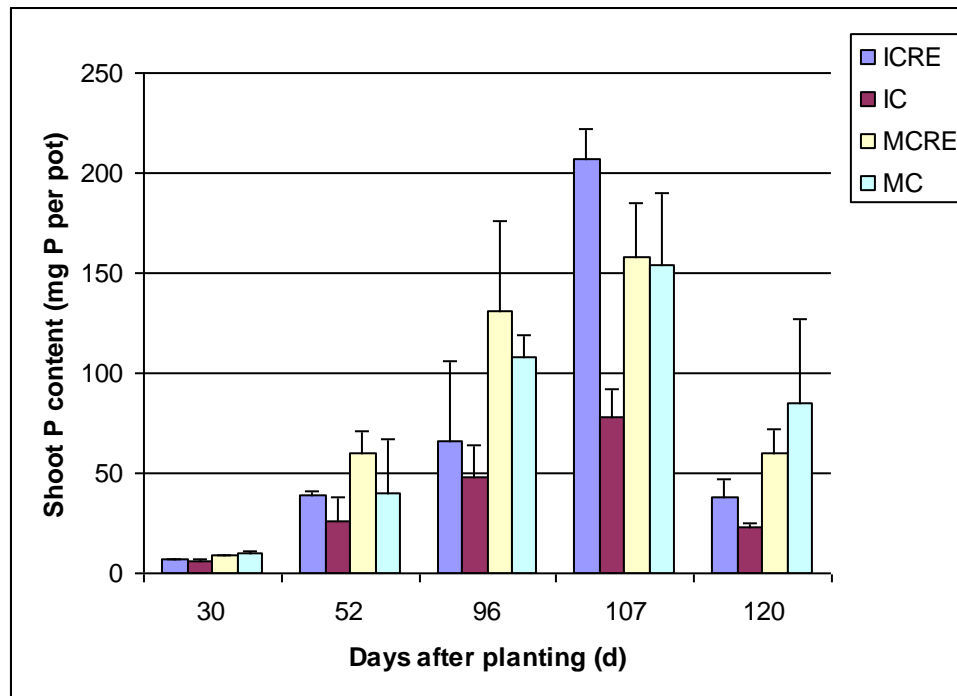


Figure 2: Shoot phosphorus content of soybean affected by root exudates (REs) in the pot experiment. ICRE - maize and soybean intercropping with REs; IC-maize and soybean intercropping without REs; MCRE-soybean monoculture with REs; and MC-soybean monoculture without REs. Mean values are followed by SD.