

## **Maize for acid soils**

### **Second scientific report -2002**

**Calba Henri\*, Jaillard Benoît\*\*, Thé Charles\*\*\*, Rojas Leyla Amparo\*\*\*\***

\*CIRAD/AMIS/Agronomie, Avenue Agropolis -BP 5035 - 34032 Montpellier

\*\* INRA-UFR de Science du Sol - 2 place Pierre Viala - 34060 Montpellier

\*\*\* IRAD, P.O. Box 2067 yaoundé, Cameroon

\*\*\*\* CORPOICA, A.A. 240142, Bogota, Colombia

## **A. INTRODUCTION**

Organic anions are implied in many reactions in the rhizosphere. The release of organic anions by roots is a mechanism frequently invoked to explain mobilisation of phosphate or detoxification of Al and the species of phosphate and citrate (or malate) are affected by the soil pH (Jones, 1996). For the low-molecular weight organic anions as citrate, the base of the reactions between the carboxyl's groups and the ligands is the formation of complex. However, these organic anions are biodegraded in a few hours in soil (Jones, 1998). In particular when amorphous Al and Fe or allophanes are present, the ligands might have direct or indirect protective effects on the organic anions and strongly slow down their degradation by micro-organisms (Boudot et al. 1989). The calibration of the functioning model of the rhizosphere requires the knowledge of the relations between the flux of the organic anions released by roots and the dynamics of Al and P between the soil and the plant. This dynamics is a function of the state of the soil exchange complex, the flux of proton release by roots and the rate of P fixation on the soil complex. In addition, it is known that citrate plays an essential role in the formation of Al complex in the rhizosphere. The calibration of the model concerned : - the effect of citrate concentration in soil solution and of soil pH on the dissolution of P, Al and Fe, and the fixation of citrate by the soil - the effect of P fixation kinetics on the P extraction by citrate.

## **B. OBJECTIVES**

The objectives for the second year of the work package 10 were to realize experimental measurements in the rhizosphere, mainly the study of the dynamics of P in the soils of Colombia and Cameroon. Effects of a citrate adding in the soil on the dynamics of P, Al and Fe in the soils were also studied.

## **C. EXPERIMENTS, RESULTS AND DISCUSSION**

### **1. Interactions between citrate and soil**

#### **1.1 Methodology**

The experiment was carried out with a soil-water ratio of 1:10, after 18 hours of equilibrium with agitation. All measurements were carried out after a contact between citrate and the soil of 1 hour in order to avoid a significant degradation of the organic acid by micro-organisms in the soil.

The purpose of the experiments was to measure:

- the effect of increasing amounts of citrate on dissolution of P, Al and Fe in soil. The experiment was carried out with 0 and 30 mg kg<sup>-1</sup> of P in Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> form.
- the effect of citrate on the P removal according to the pH of the soil with 0, 30, 100 mg kg<sup>-1</sup> of P in Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> form labelled by P<sup>32</sup> with different pH. The soil pH was adjusted between pH 4.0 and pH 6.0 per addition of HCl or NaOH 0.1M.
- the effect of the kinetics of P fixation on the extraction of P by citrate. The soils were incubated during 30 days in the presence of two amounts, 30 and 100 mg kg<sup>-1</sup> of P marked with P<sup>32</sup>, moistened to the 2/3 of the field capacity and maintained with the same moisture at a temperature of 25°C during incubation. The citrate concentrations used were 0, 0.1, 0.5, 1, 5, 10 mM.

## 1.2 Results

The results obtained with the amount of P 30 mg kg<sup>-1</sup> showed in the two soils a significant increase of Al and Fe in solution (Fig. 1). The concentration of P in solution also increased, but the concentration was 10 times lower than Al concentration. The solution samples were immediately frozen after filtration and the citrate remaining in solution was analysed later by enzymatic way. It appeared that the citrate concentration decreased in solution because of the complexation with Al and Fe, and of the adsorption on the exchange complex. The citrate concentration remaining in solution after contact with the soil is null below added citrate 0.1mM.

For Al, the results can be used to explain the differences observed between the measured and simulated quantities in the roots (see INCO1). We observed that the model over-estimated the quantities of Al accumulated in the roots. The citrate flux excreted by maize was weak, close to 100 pmol apex<sup>-1</sup> hour<sup>-1</sup> (Pellet, 1995). However, this flux is enough to maintain a gradient of concentration in the rhizosphere (Jonnes 1998), with a sphere of influence between 0.2 and 1.0 mm depending on the soil type soil, the organic anion nature and time. For example, according to Pellet (1997) the concentration of malate on the surface of the roots would range between 10 and 100µM. Our results show that between these two concentrations of citrate, the increase of Al in solution is equal to a factor 10.

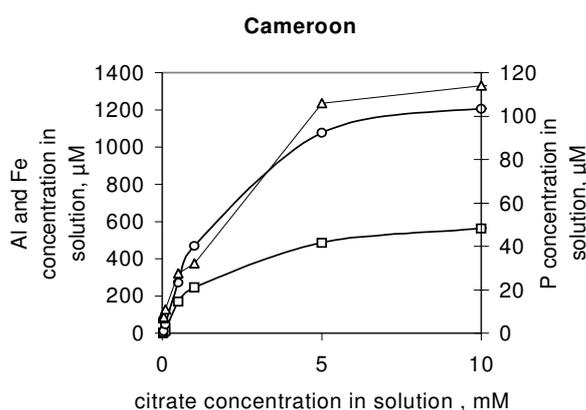


Figure 1: Al (circle), Fe (square), P (triangle) in soil solution as a function of citrate concentration in solution of Cameroon soil.

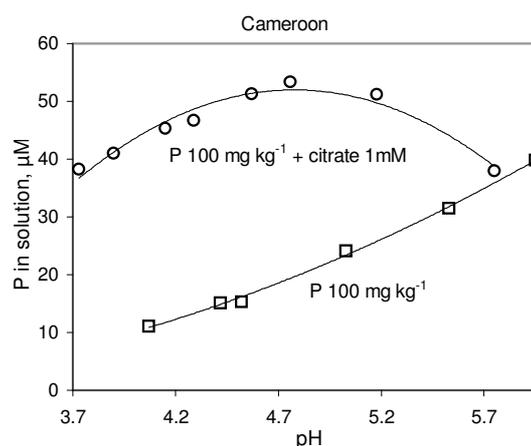


Figure 2: P in solution as a function of soil pH. Soil + 100 mg kg<sup>-1</sup> P was extract with 0 (square) or 1mM (circle) citrate.

Figure 2 shows the effect of pH on P in solution extracts with or without citrate 1mM in soils containing P 100 mg kg<sup>-1</sup>. It appeared that pH strongly decreased the solubility of P below pH 6.0. The citrate reduced P fixation on the positive charge of the exchange complex, and modified the pH effect on P fixation which resulted from the proton fixation from Al and Fe on the surface of the exchange complex.

The results obtained (fig. 3) show that the quantity of P extracted by citrate 5mM was a function of the amount of P added to the soil, and of the time of incubation. The extracted quantities of P were increasingly more significant in the soil of Cameroon at the beginning of incubation but weaker after a few days of contact. The two soils are characterized by their clay content (the soil of Cameroon is more clayey), and by their content of amorphous elements (the soil of Colombia contains more amorphous Al and Fe compounds). It appears that the extracted quantities of P vary little after 10 days of incubation. The analysis using the model will make it possible to measure the importance of the kinetics of P fixation after a minimum time of contact between the phosphate fertilizer and the soil. Figure 4 makes it possible to compare the influence of the citrate concentration in the solution of the soil on the solubilization of P for two times of contact between the soil and P, 16 hours and 30 days. The results obtained show that it is possible to linearly adjust log (P in solution) according to log (citrate) and that the line slope obtained varies little with the time of contact between the soil and phosphate. So, the relation between the two parameters can be easily implemented in the model in the form of a Freundlich equation.

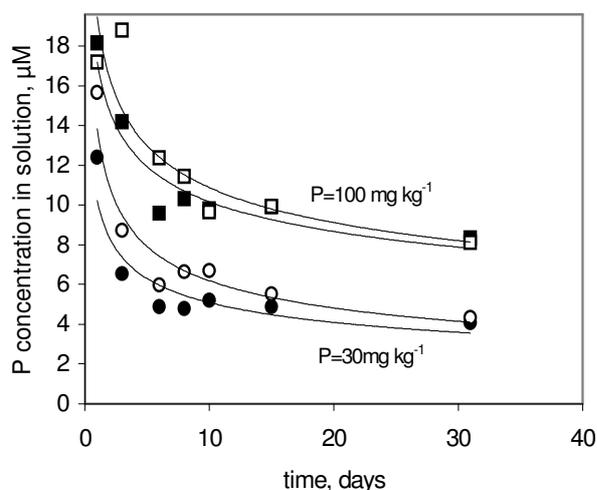


Figure 3 : P extracted by citrate 5mM as a function of time sorption of P for two levels of P added : 30 (circle) and 100 (square) mg kg<sup>-1</sup> in the soil of Cameroun (black) and Columbia (white).

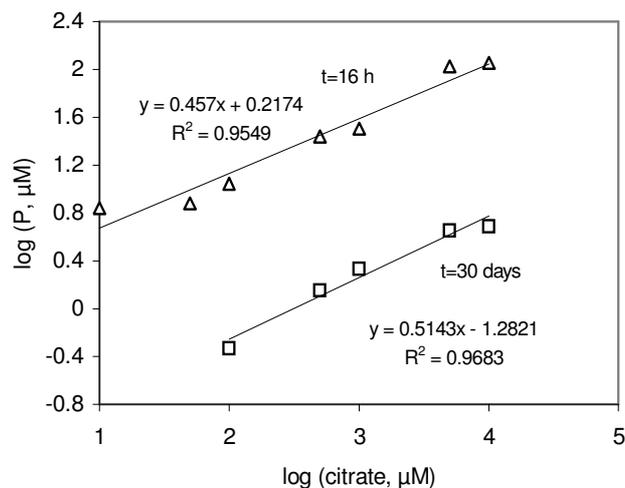


Figure 4 : Log of P concentration in solution as a function of log of citrate concentration for two time of P sorption 16 hours (triangle) and 30 days (square) in the soil of Cameroon incubated with P 30 mg kg<sup>-1</sup>.

## 4. Dynamics of P between soil and maize

### 4.1 Methodology

Al-tolerant ATP yellow (Cameroon) was used in the experiment 1. The experiment 2 consists in testing three varieties of maize, Al-tolerant ATP yellow (Cameroon), P-efficient L3x228-3 and P-inefficient 26X1113-01 (Brazil). The culture device used was adapted from Kunchenbuch and Yungk (1982) and Hinsinger (1992). It makes it possible to develop a root mat of 3mm thickness, to lay out it on a fine layer of soil also of 3mm thickness, 50mm diameter, and to carry out a cultivation of maize on the soil during a few days. The root mat was formed and used after 14 days of pre-cultivation in the higher part of the device supplied with a nutritive solution low in P (5 $\mu$ M). The soils had received 200mg kg<sup>-1</sup> (experiment 1), 100mg kg<sup>-1</sup> (experiment 2) of P in Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> form labelled by P<sup>32</sup> and were incubated 2 days before the contact with the root mat. The analyses were carried out after 8 hours, 1, 2, 3, 4, 7, 10 and 14 days (experiment 1), 14 days (experiment 2) of contact with the soil. The roots, the aerial parts of maize and the soil were analyzed separately. P<sup>31</sup> and P<sup>32</sup> were analyzed in the aerial parts after calcination and dissolution of ashes by HCl. P<sup>31</sup> and P<sup>32</sup> in the soil were analyzed after extraction 16 hours by water in a ratio 1:1.5. The relationship between the activity of P<sup>32</sup> in solution and the concentration of P<sup>31</sup> in the solution was used to calculate the fraction of P in the roots and the aerial parts coming from the soil according to the relation:

$$R_s/P_s = R_p/P_p$$

where  $R_s$  and  $R_p$  are respectively the activity of P<sup>32</sup> in the soil solution and in the plant at a given time,  $P_s$  the concentration of P in solution and  $P_p$  the fraction of P in the plant which comes from the soil.

## 4.2 Results

In the experiment 1, the activity of P<sup>32</sup> and the concentration of P in solution decreased in the water extract of the soil according to the time of cultivation. This decrease, related on the P fixation and the P remove by the plant, is faster in the case of the soil of Colombia because of its high fixing capacity for P. After 4 days of cultivation, the concentration of P in solution in the soil of Colombia reached a minimum and remain relatively constant with this level. For the soil of Cameroon, the concentration of P in solution tend towards a minimum after 8 days of cultivation. For the soil of Cameroon, the quantity of P taken by the plant accounted for 55% of P added, whereas it accounted for 28% of P added in the case of the soil of Colombia

In the experiment 2, the results show that the rhizosphere acidification depends on form of N used (NH<sub>4</sub> > NO<sub>3</sub>) and of the cultivar (Al-tolerant (ATP) > P efficient (L3-228-3) > P inefficient (26X1113-01)).

With nitrate, rhizosphere pH after cultivation was always higher than initial pH of the soils with calcium phosphate, i.e. pH 4.88 (Cameroon), pH 5.06 (Colombia). Rhizosphere pH strongly decreased with ammonium with Al-tolerant cultivar (ATPY). The cultivar P-inefficient (26X1113-01) and P-efficient (L3X228-3) did not acidify the rhizosphere with NH<sub>4</sub><sup>+</sup> but alkalinised it with NO<sub>3</sub><sup>-</sup>.

For the three varieties tested, the quantities of P taken by the roots of maize or transferred in the aerial parts decreased when the soil pH became more acid (Figure 5a). It was observed however that the quantity of P removed by the Al-tolerant cultivar was higher than that was taken by the P-inefficient or P-efficient cultivars for an identical pH close to 5.0 in the case of the Cameroon soil. The results show that the main factor explaining the removal of P by the plant was not related to efficiency with P measured with the field but on the acidification level

of the rhizosphere. In the experiment, the quantity of P removed by the plant (4.5 Mg kg<sup>-1</sup>) was very weak compared to the added quantity (100 Mg kg<sup>-1</sup>), in spite of the significant mass of root present on the surface of the soil.

The analysis of the mineral content of the roots shows generally that the Al content increases when the pH decreases. However, the Al content increases more quickly in the case of the variety P-use-inefficient. Al-tolerant cultivar accumulates less Al to pH more acid. The Al/P ratio in the root (Fig 5b) illustrates this difference. It appears in the two types of soil that the Al/P ratio in the root varies more quickly with the pH in the case of the variety P-use-inefficient. With pH 5.0, in the two soils, the Al/P ratio in the root is close to 50 for variety Al-tolerant, it is about 250 for the variety P-use-inefficient. This pH, the quantity of P removed by the plant are higher in the case of variety Al-tolerant (Fig 5b). The variety P-use-efficient is in an intermediate position.

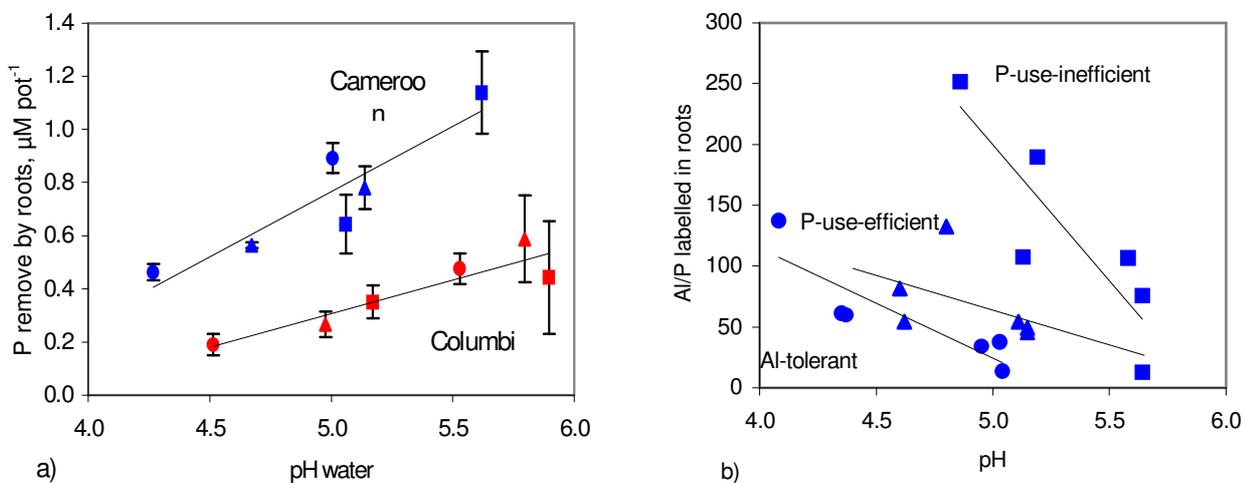


Figure 5: P remove by roots (a), ratio Al/P in root (b) of three cultivars of maize, Al-tolerant (round), P-efficient (triangle), P-inefficient (square) cultivated 14 days on Cameroon (blue) and Columbia (red) soils as a function of soil pH.

In conclusion, it appears, 1) that variety P-use-inefficient acidifies the rhizosphere less quickly, 2) that the dynamics of Al and P between the soil and the root is a function of the variety, the variety P-uses-inefficient accumulating more Al per unit of P in root remove from soil. However, the results obtained do not make it possible to conclude on the mechanisms from the inefficiency from the use of P by the variety 26X1113-01, this one and the other varieties, Al-tolerant and P-use-efficient, take P in the solution of the soil according to the bio availability of this element, defined by its soil concentration and the rhizosphere pH. In the soil the root must prospect new volumes of soil to allow the acquisition of P by the plant. The capacity of the plant to develop its root system in acid medium and to accumulate less aluminium in roots is a dominating factor for the acquisition of P by the plant. In this case, the capacity of the P-use-efficient variety to accumulate less Al in the roots constitutes a favourable factor to a better development of the root system in acid soils.

## D. GENERAL CONCLUSIONS AND SCHEDULE FOR 2<sup>ND</sup> YEAR

This second year of program made it possible to acquire the elements necessary:

- to calibrate the model. The study of the interactions between citrate and soils, Al and Fe complexation, displacement of P will make it possible to define the functions necessary to the taking into account by the model of the citrate excretion by roots
- to study the dynamics of the ions in the rhizosphere in the presence of various maize cultivars and various levels of fertilization P and to prepare the use of the experimental data obtained with the field.

## **E. LITERATURE CITED**

- Boudot, J. P., B. H. Brahim, et al., 1989. Biodegradation of synthetic organo-metallic complexes of iron and aluminium with selected metal to carbon ratios. *Soil Biol. Biochem.*, 21(7): 961-966.
- Jones, D., A. Prabowo, et al., 1996. Aluminium-organic acid interactions in acid soils. II Influence of solid phase sorption on organic acid-Al complexation and Al rhizotoxicity. *Plant and Soil*, 182: 229-237.
- Jones, D., 1998. Organic acids in the rhizosphere - a critical review. *Plant and Soil*, 205: 25-44.
- Hinsinger, P., B. Jaillard, et al., 1992. Rapid weathering of a trioctahedral mica by the roots of ryegrass. *Soil Sci. Soc. Am. J.*, 56: 977-982
- Kuchenbuch, R. and A. Jungk, 1982. A method for determining concentration profiles at the soil-root interface by thin slicing rhizospheric soil. *Plant and Soil*, 68: 391-394.
- Pellet, D. M., D. L. Grunes, et al., 1995. Organic acid exudation as an aluminium-tolerance mechanism in maize (*Zea mays* L.). *Planta*, 196: 788-795.
- Pellet, DM, Papernik La, Jones DL, Darrah PR, Grunes DL and Kochian LV 1997. Involvement of multiple aluminium exclusion mechanisms in aluminium tolerance in wheat. *Plant and Soil*, 192, 63-68.

