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THE USE OF W^0 MICRO BIOSENSOR TO MEASURE THE SITE-SPECIFIC pH CHANGES IN THE RHIZOSPHERE OF *LUPINUS SILVESTRIS* FERTILIZED WITH DIFFERENT SOURCES OF P

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ABSTRACT

Phosphorus deficient supply enhances Lupinus root secretions in the form of organic acids that lower down the soil pH. The rhizosphere pH change was determined in a green house experiment by the use of a micro-potentiometery system based on a W^0 pH electrode and $Cu/Cu(II)H_2O$ reference electrode. A linear Nikolsky type response was obtained, $y = -43.39X - 44.86$, $R^2 = 0.9962$. Total microscale acid base titrations showed the response of E= f (pH). The rhizosphere pH change was calculated during 6 weeks starting from 21 day old plants. The pH decrease by the wheat was negligible compared to that of Lupinus. The decease measured in control (P0) was prominent by Lupinus either growing alone (22.3%) or in association with wheat (25%). However, the water-soluble fertilizer (TSP) depressed the acidification process and the pH reduction was only up to 14.9%, while the sparingly soluble P compound had no effect.

Key Words: Micro-biosensor, Rhizosphere pH, Lupinus Silvestris.

INTRODUCTION

The plant roots excrete varying amounts of organic compounds in the soil; especially Lupinus emit huge quantities of organic acids under P-deficient condition (Loss et al, 1994, Brand et al, 1999; Rengel 2000; Hinnsinger, 2000; Espinosa and Hall, 2000; Horst et al, 2000; Liang and Li, 2003). The emergence of the proteoid roots structure under phosphorus deficient conditions is responsible in the excretion of varying amounts of organic acids (Marschner et al., 1987; Neumann et al., 1999). The production of these acids may lower the pH from 7.5 to 4.8 in a calcareous soil containing 20% $CaCO_3$ (Dinkalaker et al, 1989). The pH of soil sometimes brought down to as low as 3.6 (Li et al 1999). The *Lupinus arboreus* acidify the rhizosphere soil to increase the supply of orthophosphate to plant by breaking down and dissolving unavailable calcium phosphate (Espinosa and Hall 2000). The lowering of pH by the plants in calcareous soils is therefor of practical use because of its beneficial effects on the nutrient availability.

The pH measurement can be done by either collecting the rhizosphere soil and analyzing in the laboratory or can be determined by directly inserting the sensor in the rhizosphere. The former method is destructive making the instant measurement of pH difficult. The glass electrodes are not convenient to use in the microclimate of roots due to their large size. Moreover, the glass is susceptible to adsorption of organic matter that decreases the certainty of the exactitude in the measurements. Use of metal oxide sensors to determine pH (Bishop and Short, 1964; Wechter et al., 1972; Nomura and Ujihira, 1988) seems to be a good alternative to the glass electrodes. It allows to miniaturize the potentiometric system (Baeza, 2003) because of the easy conversion into wire of small diameter suitable for use in microclimate. The miniaturized metal/metal oxide sensor using W^0 wire as pH electrode and Cu wire as reference electrode is affective in the site-specific measurement of pH. Therefore, these sensors have made the nondestructive site-specific pH determination possible.

The present studies involved the observation of pH changes using W^0 sensor in the rhizosphere of *Lupinus silvestres* grown in calcimorphic soil of alkaline pH.

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MATERIALS AND METHODS

Experiment Site: The present studies were carried out partly in analytical chemistry laboratory, Department of Chemistry, UNAM, Mexico city and partly in the green house of CIMMYT, situated near Texcoco edo de Mex and in physics laboratory, Department of Edafologia, Colegio de Postgrduado, Texcoco Edo de Mex.

Soil (0-30) was brought from a field station of CIMMYT in Tlaltizapan, Morelos. The soil is alkaline classified as Ustic Petrocalcid (Rosas Calleja, 1997). The soil was air dried, ground and passed through 2mm sieve. The chemical and physical analysis was carried out for various parameters. The soil chemical characteristics determined include: pH (7.95) by potentiometer, EC (0.1 dS m^{-1}), total $CaCO_3$(19.43%) and Olsen available phosphorus (14.5 mg kg^{-1}). The soil texture was determined by the hydrometer method.

Phosphate source: Three P sources: Triple super phosphate (TSP), phosphate rock (PR) and bone meal were used (Table 1). Bone meal was achieved just by cleaning the animal bone and crushing in an electronic grinder to powder form. PR is also a non-conventional P source where as the TSP was used as a test fertilizer.

Table 1. Principle characteristics of Triple Super Phosphate (TSP), Phosphate Rock (PR) and Bone Meal (BM).

Material	Presence	% P_2O_5	%P-WS[1]	%P-CS[2]	%P-CIS[3]	ON[4]
TSP	Granular	46	85	15	------	Ca, S
PR	Powder	29.2	Traces	8.7	91.3	Ca
BM	Powder	22.9	-------	-------	------	Ca

1-water soluble; 2-citrate soluble; 3-citrate insoluble; 4-Other nutrient

Two wheat cvs (W1:Don Ernesto Inia and W2: Alondra) were cultivated in association with *Lupinus silvestres* in pots with 5 kg alkaline calcareous soil of Tlaltizapan, Morelos in a completely randomized design with three replicates, and phosphorus was applied @ 0, 40, 80 mg P Kg^{-1} as TSP, phosphate rock and bone meal.

Design for Treatments: The design of the present work contain three factors: Phosphorus sources (PS): Triple super phosphate (TSP), phosphate rock (PR) and bone meal (BM: prepared in laboratory by just grinding bones to powder form)

Phosphorus dozes (PD): 0(control) 40 and 80 mg Pkg^{-1} soil:

Crop system: two wheat cvs. W1 (Don Ernesto Inia) and W2(Alondra) grown alone and in association with Lupinus(Lupinus silvestres) and Lupinus alone. It means that we got five combinations:W1 Lup, W2 Lup, W1, W2, Lupinus

The Management of the Experiment: The plants were daily reviewed for its water requirements, to observe disease and pest infestation and to timely control any unexpected or drastic change. A basal dose of 75 mg N kg^{-1} soil as urea solution was applied after 4 weeks and 8 weeks of plants growth.

The plants were supposed to receive the same quantity of light, moisture and radiation of the sun. The plants were observed daily to make sure the presence of water at field capacity visually and manually and the deficient water was replenished on daily basis, to minimize the effects of the other factors on plant growth. The tape water was added 300 mL initially during the first 30 days increasing upto 600 mL during the last 60 days growth period. The water requirements sometimes varied depending on the climatic conditions. Wheat was grown up to maturity to get grain yield

Construction of Electrode and Micro cells for preparing Biosensor

pH Electrode: Tungsten (99% pure) wire exists in the market for its common use in welding purpose. The wire can directly be used as pH electrode.

Reference electrode: The copper wire (17.5 cm long and 0.1 cm diameter) was inserted in a plastic tube (16 cm long and 0.3 cm diameter) filled with 0.1 N KNO_3 solutions. The lower and upper end of the tube was closed with a cotton piece in such a way to isolate the internal solution of reference electrode. The Cu wire is naturally covered with a layer of Cu oxide dipped in KNO_3 solution to maintain the stable potential. The said electrode of metal-solution of quasi-referencia has been reported by Heyrovsky (1968).

Calibration of the biosensor: The pH (W^0 wire) and reference electrodes (Cu/Cu oxide) were tied together with a little rubber band and fixed in a special glass beaker along with pH meter in such a way to keep them 2 cm above the bottom. Ten mL of 0.1 N HCl was added in a beaker and titrated against 25 mL of 0.1 N NaOH adding 0.5 mL each time and noting the potential and the pH after every few seconds. The graph thus obtained, showed a relation between pH and potential (fig 1). The graph shows that E= f(pH) for the W^0 and the reference electrodes Cu/CuII. The behavior of the graph resembles the equation of Nikolsky (Esteve and Salvador, 1994) i.e., E = K – 0.06pH

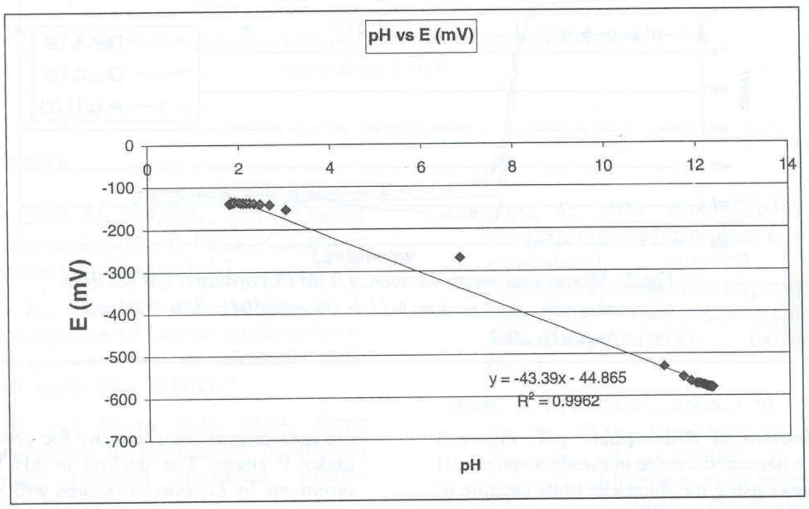

Fig 1. The calibration curve of micro biosensor obtained through titration 0.1 N HCl against 0.1 NaOH.

Determination of Rhizosphere pH: The rhizosphere pH was determined after standardizing the sensor. The pots were irrigated one day prior to recording the data to supply enough water for easy insertion of sensors and for ensuring the flow of ions in the soil. The sensor was placed close (≅ 2-3 cm) to root surface and duplicate readings were taken. The potential thus obtained was converted to pH through the calibration curve. The pH determination was exercised on weekly basis and first reading was taken at 21 days after the emergence of the plants. Simple calculations were used to find out the decrease in pH by subtracting the final value from the initial value of pH. The pH value thus obtained shows the decrease in pH. When this pH value is divided by initial pH and multiplied by 100 it gives percent decrease in pH.

RESULTS AND DISCUSSION

Relationship between potential (E) and pH: Figure 1 shows that E= f (pH) using the tungsten (W^0) as pH electrode and Cu as the reference electrode. A linear relationship was obtained that closely correspond to the equation of Nikolsky i.e., E= 54.89 mV- 49.79 pH; r^2=0.9889 showing typical behavior of the selective electrodes.

The equation shown in fig 1 is y = -43.39X – 44.86, R^2 = 0.9962 where y stands for E (mV) and X for pH and the y-axis intersect is –44.86 and slope is –43.39. The equation shows a linear relationship between potential and pH.

The construction of electrodes at different time with the same material showed the variation potentials (fig 2) during three laboratory sessions held on December 04 and 06, 2002 and August 11, 2003. The results are in accordance with the work of Baeza (2003) who worked at different times with the electrodes and got different potentials with the same initial and final volume of 0.1 N NaOH.

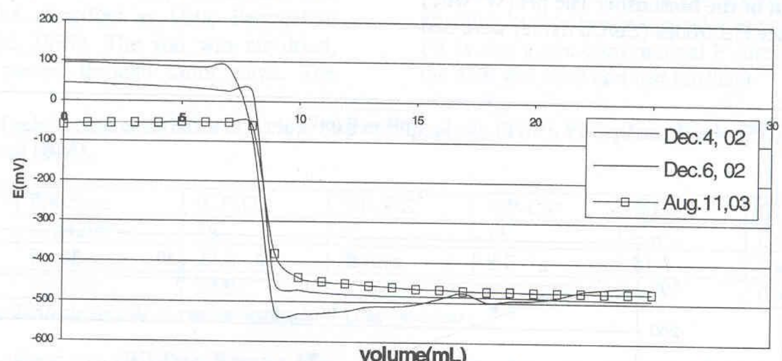

Fig. 2. Micropotentiometric titration of 0.1M HCl against 0.1 M NaOH in three aboratory sessions held on December 04 and 06, 2002 and August11, 2002.

Determination of Rhizosphere pH: Figure 3 shows the percent decrease in the rhizosphere pH in six weeks using the microelectrode capable of non-destructive site-specific measurements in the root zone. The graph shows a decrease in rhizosphere pH during the time of plant growth. The decrease was significant in *Lupinus* rhizosphere whether growing alone or in association with wheat crop. The pH decrease by the wheat was negligible compared to that of *Lupinus*. The percent pH reduction was greater (25%; from pH 7.76 to 5.82) in the root zone of *Lupinus* associated with wheat (Don Ernesto Inia) followed by the *Lupinus* alone (22.3%; pH7.63 to 5.93) without the external application of phosphorus (control). The results are in agreement with Dinkalaker et al., (1989) and Loss et al (1994) who attributed the decrease to the excretion of citric acid by the proteoid roots under P stress. The decline in pH was again prominent by *Lupinus* associated with wheat (DE inia) with value of 23% (pH7.63 to 5.88) and by *Lupinus* growing alone (22.5; from pH 7.8 to 6.05) at the application of 40 mg P kg^{-1} as bone meal (BM40) and rock phosphate (RP40), respectively. However, the acidification was depressed on the application of the external water-soluble fertilizer as highest dose of triple super-phosphate (TSP80), the pH decline was limited upto 14.9 % (from pH 7.65 to 6.5). The results are in agreement with Marschner (1986) and Watt and Evans (2003) who found that fertilization with soluble P depressed the formaton of the proteoid roots, which are responsible for the production of the organic acids.

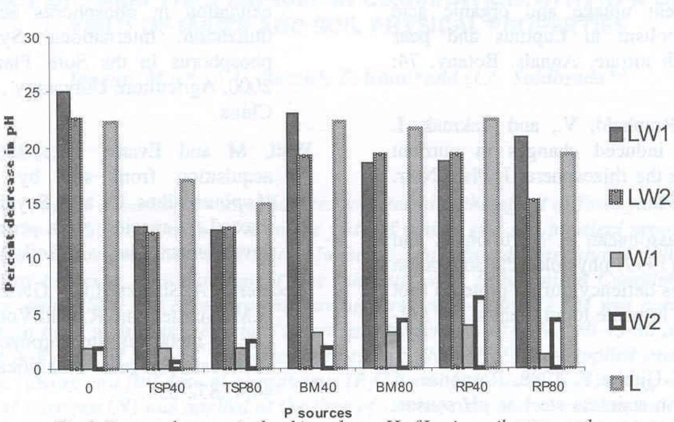

Fig. 3. Percent decrease in the rhizosphere pH of Lupinus silvestres and wheat during 6 weeks.

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