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Full Length Research Paper

Changes in selected soil chemical properties in the rhizosphere of *Phaseolus vulgaris* L. supplied with *Rhizobium* inoculants, molybdenum and lime

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Selected rhizosphere soil chemical properties were studied to establish changes in soil fertility status in response to *Rhizobium* inoculation in *Phaseolus vulgaris* L., molybdenum and lime supply after the growing season. The experiment was laid in a split-split plot and consisted of 2 levels of *Rhizobium* inoculation (with and without rhizobia), 3 levels of Mo (0, 6 and 12 g kg^{-1} of seeds) and 3 levels of lime (0, 2 and 3 t ha^{-1}). *Rhizobium* inoculation showed significant increase in the soil pH, Ca and Na. The available micronutrients Fe, Cu, Zn and Mn were also significantly increased in the *Rhizobium* inoculated treatments compared with the uninoculated controls. Molybdenum and lime supplied individually significantly increased the soil pH. Furthermore, Ca and Mg levels in the soil were significantly increased with lime. Interactive effects were found between *Rhizobium* and Mo on pH changes in the rhizosphere. *Rhizobium* inoculation, liming and Mo application are the strategies used to appropriately supply nutrients to the plants. In this study, inoculation significantly increased the soil pH and the availability of Ca, Na, Fe, Cu, Zn and Mn in the rhizosphere. The increased available nutrients due *Rhizobium* inoculation could be due to increased soil pH condition to near neutral values. Additions of lime resulted in increased soil pH and exchangeable Ca and Mg. Repeated applications of these inputs in the study area is recommended because soils are acidic in nature.

Key words: Calcium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, soil pH, zinc.

INTRODUCTION

Rhizobium is also known as bio-fertilizer (Alaa El-Din et al., 1985) as they may increase the availability of soil nutrients to the plants and in the rhizosphere through processes such as biological N_2 fixation. They may also contribute to soil nutrition from their dead cells (McCulley, 2001) or making nutrients more available through solubilisation of phosphates and other minerals bound in unavailable forms such as Fe. Studies have reported the solubilisation of P in the rhizosphere by *Rhizobium leguminosarum* bv. *Phaseoli* (Chabot et al., 1998). For instance, in a nodulated fixing pigeon pea plant, P availability was enhanced by the legume through release of piscidic acid in pigeon pea root exudates, which mobilised and increased P availability (Ae et al., 1990). Other mechanisms are related to siderophore production which helps facilitate the solubilisation of certain nutrients such as Fe from unavailable to more available form (Dakora and Phillips, 2002). There is research evidence that some rhizospheric bacteria produce siderophores which solubilise Fe (Bar-Ness et al., 1991; Dakora and Phillips, 2002; Wang et al., 1993).

Nitrogen fixed by the rhizobial in the host plant may be released into the rhizosphere through the root exudation (Ndakidemi, 2006; Eaglesham et al., 1981) and thus improving the N status of the soil. Plants fixing nitrogen may also manifest changes in soil pH (Bolan et al., 1991; Nye, 1981) and other physical and chemical

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characteristics such as the level of plant exudates (Griffiths et al., 1999; Revsbech et al., 1999; Tavaria and Zuberer, 1998; Uren and Reisenauer, 1988; Xu, 2000).

Research evidence on the effect of lime on soil chemical properties is widely available in the literature (Meda et al., 2002; Meiwes, 1995). For instance, lime is reported to play a key role in raising the soil pH and increasing the exchangeable Ca and Mg in the soil (Meiwes, 1995).

Besides the availability of adequate information on the effects of *Rhizobium* inoculation on N economy of soils, very few studies have assessed their impact on the availability of other nutrient elements in the rhizosphere of legumes. This study examines the effects of i) *Rhizobium* inoculation, ii) Mo supply and iii) lime application on the concentrations of plant-available nutrients in the rhizosphere of *Phaseolus vulgaris*.

MATERIALS AND METHODS

Experimental site

The experiments were conducted in the glasshouse of the Cape Peninsula University of Technology (CPUT), Cape Town Campus, Keizersgracht from October 2008 to December 2008. Field experimentation was also conducted under irrigation at the Agricultural Research Council Nietvoorbij site (33°54'S, 18°14'E) in Stellenbosch, South Africa, during the summer seasons, from October 2008 to March 2009. The site lies in the winter rainfall region of South Africa at an elevation of 146 m above sea level. The mean annual rainfalls on the farm is 713.4 mm and mean annual temperatures range from 22.6°C (day temperature) to 11°C (night temperature).

The soil type was sandy loam classified as Glenrosa, Hutton form (SCWG, 1991), equivalent to skeletic leptosol in the FAO soil classification system (FAO, 2001). Following land preparation, but prior to planting, soil samples were collected for nutrients analysis.

Experimental design

The experimental treatments consisted of 2 levels of *Rhizobium* inoculation (with rhizobia and without rhizobia), 3 levels of Mo (0, 6 and 12 g per kg of seeds) and 3 levels of lime (0, 2 and 3 t ha^{-1}). The experimental design followed a spilt-split-plot design with 4 replications per treatment. The field plots measured 2.5 x 4 m with 5 rows 0.5 m apart from one another. *P. vulgaris* was sown with inter-row planting distance of 20 cm. The plots were interspaced by small terraces of 1 m to prevent contamination. The plant population density was 200,000 plants ha^{-1}.

Planting was done after ploughing and harrowing. Lime application was done 2 weeks before planting. 12 h before planting, seeds were soaked into Mo solution. The zero Mo control was also soaked in a water solution containing no Mo. To avoid contamination, all *Rhizobium* uninoculated treatments were sown first. *Rhizobium* inoculation was done manually by putting the inoculant (*Rhizobium leguminosarum* biovar phaseoli-bakteriee registrasienr. L1795 wet 36/1947) in the planting hole. The inoculants used were obtained from University of Pretoria, South Africa.

Collection and preparation of bulk soil

Soil samples were collected with auger (0 - 20 cm depth) from several locations within each replicate plot and mixed for determination of the initial nutrient concentrations in the soil. The soil samples were air-dried in the laboratory and sieved (2 mm) for analysis of nutrients and determination of pH and organic matter.

Collection and preparation of rhizosphere soil

At 60 days after planting (DAP), rhizosphere soil, defined as rich in roots and/or adhering to the roots and influenced by root activity, was collected from around *P. vulgaris* plants for nutrient analysis. To achieve this, soil was carefully excavated from around single plants or their pairs down to 30 cm or more (depending on root depth) and the island of soil around the plant dug up and removed, with the plant and its roots intact inside the lump of soil. Using one's hands, the volume of soil containing intact plant(s) was removed from the exterior down to a root-rich rhizosphere soil material of about 30 - 50 g. This sample was shaken into a labelled plastic bag and the process repeated for up to 72 samples. These rhizosphere soil samples were air-dried in the laboratory and sieved (2 mm) for analysis of nutrients and the determination of pH and plant-available nutrients in rhizosphere soil.

Measurement of soil pH

The pH of soil was measured in 0.01 M $CaCl_2$ solution using a 1:2:5 soil-to-solution ratio.

Determination of plant-available nutrients in rhizosphere soil

The extractable P, K, Na, Ca and Mg were determined by citric acid method as developed by Dyer (1894) and modified by the Division of Chemical Services (DCS, 1956) and Du Plessis and Burger (1964). A 20 g air-dried soil sample was extracted in 200 mL of 1% (w/v) citric acid, heated to 80°C, shaken for 2 min at 10 min intervals over a total period of 1 h and filtered. A 50 mL aliquot was heated to dryness on a water bath and 5 mL of concentrated HCl and HNO_3 and 20 mL of deionised water added. The mixture was heated to dissolve the dry residue and the sample filtered. Measurements of P, K, Na, Ca and Mg were done directly by direct aspiration on the calibrated simultaneous ICP.

The trace elements and Cu, Zn, Mn and Fe were extracted from soil using di-ammonium ethylenediaminetetraacetic (EDTA) acid solution Trierweiler and Lindsay (1969), as modified by Beyers and Coetzer (1971). The extractants were analysed for Cu, Zn, Mn and Fe using the calibrated simultaneous ICP spectrophotometer.

Statistical analysis

The data from this experiment was analyzed using the software of STATISTICA program 2008 (StatSoft Inc., Tulsa, OK, USA). Fisher's least significant difference was used to compare significant treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

RESULTS

Effects of *Rhizobium*, molybdenum and lime on pH of rhizosphere soil of *Phaseolus vulgaris* L.

Rhizobium inoculation, Mo and lime significantly increased the rhizosphere pH of *P. vulgaris* (Table 1). For

Table 1. Effect of *Rhizobium*, molybdenum and lime on macro-nutrients measured in field during 2008 - 2009 season.

Treatments	pH	P	K	Ca	Mg	Na
			mg kg^{-1}			
Rhizobium						
-R	6.2±0.0b	5.1±0.1a	105.5±2.7a	3.5±0.3b	2.4±0.2a	79.1±2.0b
+R	6.4±0.1a	5.8±0.5a	112.5±3.5a	4.8±0.3a	2.3±0.3a	85.4±2.0a
Molybdenum (g.kg^{-1})						
0	6.1±0.0b	6.0±0.7a	107.9±4.0a	3.9±0.4a	2.3±0.3a	82.5±2.6a
6	6.3±0.0ab	5.3±0.3a	109.1±4.0a	4.0±0.4a	2.3±0.3a	80.8±2.6a
12	6.5±0.1a	5.2±0.3a	110.0±3.6a	4.5±0.4a	2.5±0.3a	83.4±2.4a
Lime (t.ha^{-1})						
0	6.1±0.0b	5.1±0.4a	110.2±3.4a	3.1±0.3c	1.3±0.2c	84.4±2.4a
2	6.3±0.1ab	5.6±0.6a	109.1±4.5a	4.0±0.4b	2.2±0.2b	82.1±2.2a
3	6.4±0.1a	5.8±0.4a	107.6±3.7a	5.3±0.4a	3.6±0.2a	80.2±2.9a
3-Way ANOVA F-statistic						
R	15.4***	1.28 NS	2.1 NS	10.6**	0.1 NS	4.7 *
Mo	43.1***	0.70 NS	0.06 NS	0.8 NS	0.2 NS	0.275
L	20.3***	0.46 NS	0.10 NS	9.7***	25.4***	0.678 NS

-R, without *Rhizobium*; +R, with *Rhizobium*. Values presented are means ± SE. **, *** = significant at P ≤ 0.01, P ≤ 0.001 respectively, NS = not significant. Means followed by similar letter (s) in a column are not significantly different from each other at P ≤ 0.05.

instance, *Rhizobium* inoculation increased the rhizo-spherepH by 3.2% compared with the uninoculated control. Molybdenum at 6 and 12 g kg^{-1} of seeds increased significantly the soil pH by 3.2 and 6.5% compared with the zero Mo control. Furthermore, lime at 2 and 3 t ha^{-1} increased significantly the rhizosphere pH by 3.2 and 4.9% respectively relative to zero lime control treatment (Table 1).

Effects of *Rhizobium*, molybdenum and lime on macronutrient concentrations in rhizosphere soil of *P. vulgaris*.

The macronutrient concentrations (P, K and Mg) were not significantly affected by Rhizobium inoculation. However, Ca and Na were significantly increased by *Rhizobium* inoculation. *Rhizobium* inoculation increased Ca by 37 and Na by 78% compared with the uninoculated control (Table 1).

Molybdenum had no significant effect on any of the macronutrient concentrations in rhizosphere soil of *P. vulgaris*.

Application of lime at 2 and 3 t ha^{-1} resulted into significant increase in Ca and Mg. In this study, the level of Ca and Mg in the rhizosphere of *P. vulgaris* increased by 29 and 71% for the Ca and 69 and 180% for Mg compared with the control (Table 1).

Effects of *Rhizobium*, molybdenum and lime on micronutrient concentrations in rhizosphere of *Phaseolus vulgaris* L.

The result in Table 2 indicates that *Rhizobium* inoculation significantly increased the concentrations of only Cu, Zn, Fe and Mn in the rhizosphere of *P. vulgaris* L. compared with the control. For example, the concentrations of Cu, Zn and Fe increased respectively by 20, 67 and 28% with *Rhizobium* inoculation compared with the control (Figure 1).

Molybdenum and lime had no significant effect on all micronutrient concentrations in rhizosphere of *P. vulgaris* L. (Table 2).

Interactive effect of *Rhizobium*, molybdenum and lime on the concentration of nutrients in the rhizosphere of *Phaseolus vulgaris* L.

There was an interactive effect between *Rhizobium* and molybdenum only for pH values in the rhizosphere soil of *P. vulgaris* (Figure 2). *Rhizobium* inoculation combined with Mo gave significantly higher pH values compared with all other treatments (Figure 2).

DISCUSSION

Rhizobium inoculation in this study significantly decreased

Table 2. Effect of *Rhizobium*, molybdenum and lime on micro-nutrients measured in the field during 2008 – 2009 season.

Treatments	Fe	Cu	Zn	Mn
		*u*g kg^{-1}		
Rhizobium				
-R	155.9±6.3b	1.5±0.1b	0.6±0.0b	27.4±6.3b
+R	199.9±9.6a	1.8±0.1a	1.0±0.1a	30.5±7.5a
Molybdenum (g.kg^{-1})				
0	174.2±9.8a	1.7±0.1a	0.8±0.1a	21.7±6.2a
6	174.7±10.2a	1.7±0.1a	0.8±0.1a	32.6±9.2a
12	184.8±12.7a	1.7±0.1a	0.8±0.1a	32.6±9.4a
Lime (t.ha^{-1})				
0	176.6±10.1a	1.8±0.1a	0.8±0.1a	27.3±8.3a
2	175.5±10.0a	1.7±0.1a	0.8±0.1a	29.7±8.6a
3	181.6±12.7a	1.6±0.1a	0.8±0.1a	29.8±8.6a
3-Way ANOVA F-statistic				
R	11.9**	7.3**	8.4**	0.08 NS
Mo	0.30 NS	0.03 NS	0.07 NS	0.45 NS
L	0.09 NS	0.67 NS	0.15 NS	0.02 NS

-R, without *Rhizobium*; +R, with *Rhizobium*. Values presented are means ± SE.
, * = significant at P ≤ 0.01, P ≤ 0.001 respectively, NS = not significant. Means followed by similar letter (s) in a column are not significantly different from each other at P ≤ 0.05.

decreased the soil acidity by increasing the soil pH in the rhizosphere of *P. vulgaris* (Table 1). These are positive results especially in acidic soils where low pH is responsible for poor plant growth (Meiwes, 1995) which limits the uptake of some important nutrients such as phosphorous (Dakora and Phillips, 2002) and the decomposition of organic materials in the soil (Motavalliet al., 1995). Improved soil pH to optimum levels from different practices is advantageous as may improve the soil chemical properties and the availability of certain mineral nutrients in the soil (Bagayoko et al., 2000; Condron et al., 1993; Table 1) and hence the plant growth. The mechanism involved in *Rhizobium* inoculation improving pH of the soil is complex, but research evidence has shown that plants that absorb nitrogen as NO_3^- tend to raise the pH in the rhizosphere (Dakora and Phillips 2002; Nye, 1981) a phenomenon which was not proved in this study.

Rhizobium inoculation numerically but not significantly increased the concentrations of P and K. However, the rhizosphere concentrations of Ca, Na (Table 1), Fe, Cu, Zn and Mn (Figure 1) were significantly increased with *Rhizobium* inoculation. It is not well established on how *Rhizobium* makes these mineral nutrients more available in the rhizosphere, but few possible options are proposed. Firstly, there is evidence that *Rhizobium* can increase the availability of nutrients such P and Fe through a mechanism involving their solubilisation from unavailable to available forms (Chabot et al., 1998; Dakora and Phillips, 2002). Secondly, certain rhizospheric bacteria may produce siderophores which solubilise Fe (Bar-Ness et al., 1991; Dakora and Phillips, 2002; Wang et al., 1993) and makes it available into soil solution for plant uptake, a scenario which was supported by results from this study. Thirdly, the decaying rhizobial cells could also increase the nutrient availability in the rhizosphere, an argument supported by McCulley (2001). Lastly, it is possible that soil inoculated with *Rhizobium* may have increased their biological activity associated with roots of the host plant and the micro-organism, thus increasing the decomposition of organic matter in the soil, root-residue decomposition enhanced root exudation and hence increasing the amount of available nutrients in the soil (Eskelinen et al., 2009; Hauggaard-Nielsen and Jensen, 2005; Lee and Pankhurst 1992; Murphy et al., 2004; Sanon et al., 2009). Whatever mechanism employed, *Rhizobium* inoculation seemed to be very beneficial in increasing some mineral elements in the soil. Molybdenum application at 6 and 12 g kg^{-1} of seeds significantly increased the soil pH relative to the control treatment (Table 1). Noticeable increases were reported by supplying Mo at 12 g kg^{-1} of seeds. The mechanisms involved are not clearly understood, but it is possible that Mo stimulated N-fixation and uptake and metabolism

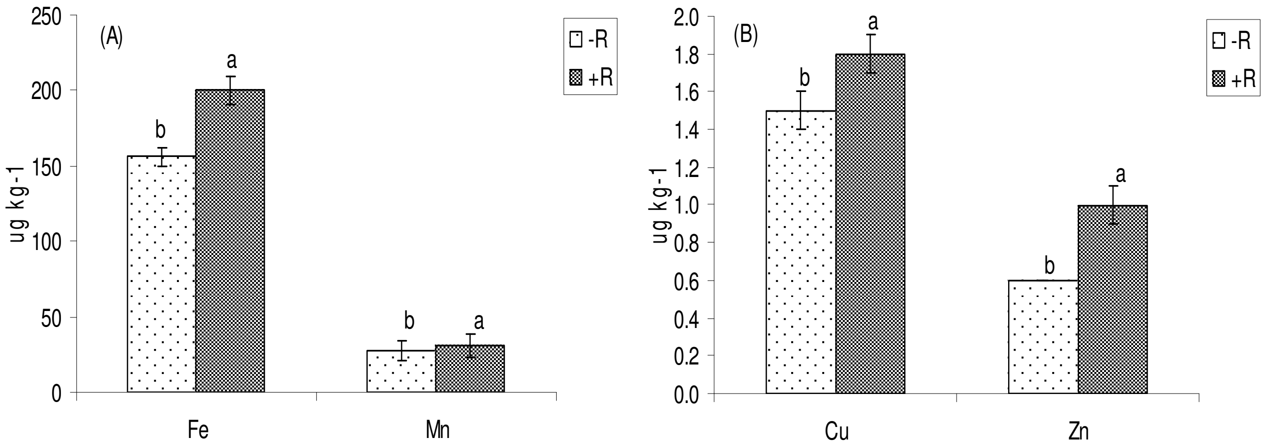

Figure 1. Effect of *Rhizobium* on soil micronutrients: A) Fe and Mn, B) Cu and Zn

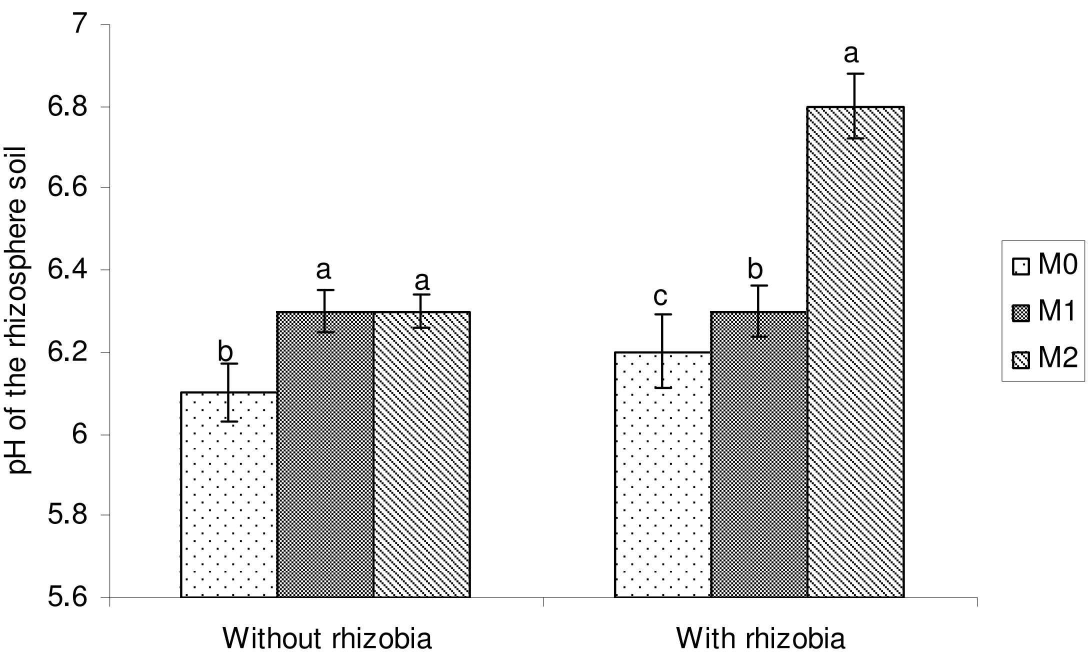

Figure 2. Interaction effect of *Rhizobium* and molybdenum on the pH of rhizosphere soil on field experiment in 2009.

of nitrates which ultimately resulted into the soil pH increase (Nye, 1981).

As expected, application of lime at 2 and 3 t ha^{-1} resulted into significantly increased soil pH and the available Ca and Mg relative to the control treatment. Liming is done in acidic soils to reduce acidity (Okpara et al., 2007; Jessop and Mahoney, 1982) and to supply important plant nutrients such as Ca or Mg (Steiner and Alderman, 2003; Pierce and Warncke, 2000). As the used study site was previously reported to be deficient in Ca and Mg (Ndakidemi, 2005), application of lime was justifiable as it improved the soil pH and the basic cations: Ca and Mg into the rhizosphere.

Conclusion

In conclusion, this study found that *Rhizobium* inoculation significantly increased the soil pH and the availability of Ca, Na, Fe, Cu, Zn and Mn in the rhizosphere. The increased available nutrients due *Rhizobium* inoculation could be due improved favourable pH conditions of near neutral values. Additions of lime resulted in increased soil pH and exchangeable Ca and Mg. The treatments involving the combination of *Rhizobium* and Mo at the highest supply rate resulted into significant interactions and gave the excellent pH changes near to neutral.

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