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Rhizobium inoculation and the supply of molybdenum and lime affect the uptake of macroelements in common bean (*P. vulgaris* L.) plants.

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Abstract

This study was conducted to investigate the effect of *Rhizobium* inoculation and the supply of Mo and lime on the uptake of macroelements in *P. vulgaris*. To achieve this aim, experiments were conducted at the glasshouse of the Cape Peninsula University of Technology, Cape Town (i.e. from August 2008 to January 2009) and field experiment at the Agricultural Research Council (ARC) Nietvoorbij site in Stellenbosch, South Africa (i.e. during the summer seasons from October 2008 to March 2009). A randomised complete block design was used in a 3-factorial arrangement with 2 levels of *Rhizobium* inoculation (with and without rhizobia), 3 levels of Mo (0, 6 and 12 g kg^{-1} seeds) and 3 levels of lime (0, 2 and 3 t ha^{-1}) with 4 replications per treatment. The result showed that *Rhizobium* inoculation significantly ($P \leq 0.05$) increased the uptake of P, K, Ca and Mg in the plant parts attributed to increased soil pH. Similarly, Mo significantly ($P \leq 0.05$) increased the uptake of P, K, Ca and Mg in all organs of *P. vulgaris* but decreased the S content in roots. Application of lime significantly increased the uptake of the macroelements in shoots and whole plant only in the glasshouse probably due to differences in soil volumes between the glasshouse and the field conditions. There was significant interaction between *Rhizobium* × Mo on P, K and S in roots, shoots and pods of *P. vulgaris* in glasshouse and field experiments; *Rhizobium* × lime on the amount of S in shoots and whole plant of *P. vulgaris* grown in the field experiments; Mo × lime on the uptake of P in roots and Ca in pods in glasshouse as well as P in roots in field studies. Regardless of the type of interaction, combining *Rhizobium* and Mo at 12 g g^{-1} seeds and/or lime at 3 t ha^{-1} maximised the uptake of P and K in roots and Ca in pods compared with zero control. These results suggest that *Rhizobium* inoculation and the supply of Mo and lime can be of great benefit to farmers growing grain legumes such as *P. vulgaris* in acidic soils ranging from pH 6.1 to 6.2. Furthermore, these inputs have shown to improve the uptake of P, K, Ca and Mg, which are greatly required by the plant for growth, nodulation and N_2 fixation.

Keywords: amelioration, amount, legumes, liming, plant nutrients, soil acidity.
Abbreviations: ANOVA-Analysis of variance; ATPases-Adenosine triphosphatases; Ca-Calcium; Chl-Chlorophyll; DAP-Days after planting; IAA-Indole 3-acetic acid; K-Potassium; Mg-Magnesium; Mo-Molybdenum; N_2-Nitrogen; P-Phosphorus; pH-Soil reaction; *P vulgaris-Phaseolus vulgaris*; RNA-Ribonucleic acid; RuBP-Ribulose-1, 5-bisphosphate; S-Sulphur.

Introduction

Bioavailability and uptake of primary and secondary macroelements such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) is very important for plant growth and development in Africa where different cropping systems involving legumes such as common beans (*Phaseolus vulgaris* L. variety Provider – purchased from Rwanda) is practiced. Their uptake by plants depends largely on the amount, concentration and activities in the rhizosphere soil as well as the capacity of the soil to replenish them in the soil solution. Specifically, P is essential for plant growth and function including symbiotic N_2-fixation processes (Sa and Israel, 1991). Calcium is involved in cell wall structure, membrane structure and function, optimal root hair colonization, signal-response coupling, early infection events, induction of high number of nodules and synthesis of flavonoids (Rudd and Franklin-Tong, 2001; Sanders et al., 2002). Magnesium is involved in numerous physiological and biochemical processes in plants such as ATPases, ribulose-1,5-bisphosphate (RuBP) carboxylase, RNA polymerase and protein kinases and plays a central role atom of the Chl molecule (Shaul, 2002; Epstein and Bloom, 2004). Potassium is essential for many physiological processes, such as photosynthesis, translocation of photosynthate into sink organs, activation of enzymes, and reducing excess uptake of ions in stressed abiotic environment (Mengel and Kirkby, 2001). Sulphur is a constituent of several plant biochemicals which regulate plant growth and also plays an important role in N_2 fixation (Beinert, 2000; Marquet, 2001; Noctor et al., 2002; Kadıoğlu, 2004). Sulphur is equally essential in the synthesis of chlorophyll and in the electron movement in the photosynthesis process (Sanda et al., 2001; Kadıoğlu, 2004). Along with Mg, S plays a role in the formation of oils within the seeds. Acid soils such as those found in Africa are often characterized by low bioavailability of some plant nutrients including P, K, Ca, Mg and S due to low soil pH or related phytotoxicity problems ascribed to H^+ and Al^{3+} ions (Marschner, 1991). Globally, about 40% of cultivated soils have acidity problem leading to significant decreases in crop

production (Sumna and Noble, 2003). For example, Hanson (1992) reported that of the 3 billion hectares of arable land in tropical Africa, 16.9% has high soil acidity, and 6.8% has high P fixation. In order for the legume plants such as *P. vulgaris* to establish and grow successfully on such acid soils, strategic management such as molybdenum (Mo) and liming supply is required in order to effectively overcome or minimize soil acidity and related phytotoxicity and/or nutrient deficiencies prevailing on these soils. Common beans are considered to be the most important grain legume for direct human consumption; and comprise 50% of the grain legumes consumed worldwide (Broughton et al., 2003; Graham et al., 2003). In Africa, *Phaseolus vulgaris* is a staple crop serving as a direct primary source of protein in the diet. However, low capacity to nodulate and N_2 fixation as well as environmental factors such as low macroelements content in the soil due to acid soil conditions are important constraints for the common bean production in most areas of Africa where the crop is grown (Broughton et al., 2003; Graham et al., 2003). Although several studies have been conducted on the effect of *Rhizobium*, Mo and lime on a number of factors on *P. vulgaris* (Bambara and Ndakidemi, 2010a, b), limited studies are available on the effect of these factors on mineral elements uptake in *P. vulgaris*. This study investigated the effect of *Rhizobium* inoculation and the supply of Mo and lime on the uptake of macroelements (P, K, Ca, Mg and S) in *P. vulgaris*.

Results

Effects of Rhizobium inoculation on the amount of macroelements in the plant organs of P. vulgaris.

There were significant differences on the uptake of P, K, Ca, Mg and S in the roots, shoots, pods and whole plant of *P. vulgaris* inoculated with *Rhizobium* in the glasshouse and field experiments (Table 1). For example, inoculation of *P. vulgaris* with *Rhizobium* significantly ($P \leq 0.05$) increased the amounts of Ca, Mg and S in roots. Although the amount of P and K increased in the root as a result of *Rhizobium* inoculation, these values were only significant in the glasshouse experiment but not in the field experiment. The result also showed that the uptake of P, K, Ca, Mg and S in shoots, pods and whole plant were significantly greater in inoculated compared with the uninoculated treatment in glasshouse and field experiments (Tables 1, 2, 3 and 4).

Effects of molybdenum on the uptake of macroelements in the plant organs of P vulgaris

Supplying Mo at 6 and 12 g kg^{-1} of seeds in glasshouse and field experiments significantly increased the uptake of P, K, Ca and Mg in roots, shoots, pods and whole plant *P. vulgaris* compared with the control (Tables 1, 2, 3 and 4). However, although the supply of Mo at 6 and 12 g.kg^{-1} of seeds significantly decreased the amount of S in roots; in shoots, pods and whole plant, the amount of S was not significantly changed in both glasshouse and field experiment (Tables 1, 2, 3 and 4).

Effects of lime supply on amounts of macroelements in the plant organs of P vulgaris

The effect of lime supply on the macroelements uptake in the roots, shoots, pods and whole plant of *P. vulgaris* is shown in Tables 1, 2, 3 and 4. Relative to control, supplying lime at 2 and 3 t ha^{-1}, the amounts of P, K, Ca and Mg in shoots and whole plant were significantly greater in glasshouse but not in field experiment (Tables 3 and 4). However, similar supply of lime in glasshouse and field experiments significantly decreased the uptake of S in roots, shoots and whole plant (Tables 1, 2 and 4) but not in roots (i.e. glasshouse experiment) and in pods where the amount of S was not significantly changed (Table 3).

Differences on the uptake of macroelements between field and glasshouse experiments

There was a difference on the uptake of microelements between field and glasshouse experiments. Whether inoculated with *Rhizobium*, supplied with Mo or lime, the uptake of macroelements was greater in the field compared experiment with the glasshouse experiment (Tables 1, 2, 3 and 4)

Interactions

There was significant interaction between *Rhizobium* × Mo on P, K and S in roots, shoots and pods of *P. vulgaris* in glasshouse and field experiments (Figs. 1 and 2). Without *Rhizobium* inoculation, supplying Mo significantly increased the uptake of P and K in roots, S in shoots and pods in the glasshouse and field experiments (Figs. 1A, B, C and D; 2A, B and C). However, combining *Rhizobium* inoculation with Mo, the uptake of P and K were significantly increased in roots (Figs. 1A and B) but not S in shoots and pods which significantly showed a declining trend (Figs. 1C, D and 2C) compared with un-inoculated treatment. With the exception of S, significantly greater uptake of these macroelements was observed when Mo was applied at 12 g kg^{-1} of seeds.

The data also showed significant interactive effect of *Rhizobium* × lime on the amount of S in shoots and whole plant of *P. vulgaris* grown in the field experiments (Fig. 3A and B). Although supply of lime without *Rhizobium* did not indicate any significant change, combining *Rhizobium* inoculation with lime, significantly decreased the uptake of S in shoots and whole plant.

Significant interactive effects of Mo × lime was also observed on the uptake of P in roots and Ca in pods in glasshouse (Figs 4A, B) as well as P in roots in field studies (Fig. 4C). Results showed that combining Mo with lime significantly increased the amount of P in the roots and Ca in pods relative to zero control. From these interactions, combining Mo at 12 g kg^{-1} of seeds and lime at 3 t ha^{-1} maximised the uptake of P in roots and Ca in pods compared with the zero control.

Discussion

Fertility and productivity of soils in different parts of the world is rapidly declining due to various forms of degradation including soil acidity (Gruhn et al., 2000; Cakmak, 2002). These degradations have caused growth-limiting problems associated with mineral-elements deficiencies and toxicities in about 40 - 60% of the arable soils (Cakmak, 2002; Okalebo et al., 2009) and relative crop yields reduction associated with such abiotic stress vary between 54% and 82% (Bray et al., 2000). *Rhizobium*, a soil microorganism which is capable of establishing symbiotic relationship with legumes such as the *P. vulgaris*, can fix

Table 1. Effect of with and without *Rhizobium*, Mo and lime supply on the macroelements uptake in roots of *P. vulgaris* L. as measured in the glasshouse and field.

Treatments	Glasshouse experiment					Field experiment				
	P	K	Ca	Mg	S	P	K	Ca	Mg	S
			mg plant^{-1}					mg plant^{-1}		
Rhizobium										
R-	0.7±0.1b	8.2±0.9b	5.6±0.7b	2.0±0.2b	2.0±0.1b	10.5±1.5a	128.1±17.0a	86.1±13.0b	30.6±3.2b	29.0±2.1b
R+	0.8±0.1a	9.7±0.5a	7.9±0.5a	2.7±0.3a	2.4±0.1a	11.8±0.9a	149.2±8.2a	122.3±7.2a	42.1±4.5a	38.6±2.7a
Molybdenum (g kg^{-1})										
0	0.5±0.1b	6.4±0.7b	4.7±0.5c	1.6±0.2b	2.4±0.2a	8.3±1.2b	106.2±14.4b	78.3±10.8b	26.7±3.6b	39.4±3.5a
6	0.7±0.1b	9.2±0.8b	6.7±0.5b	2.3±0.2a	2.4±0.1a	10.4±1.1b	140.8±15.7a	99.9±9.4b	34.4±3.0b	34.9±2.6a
12	1.0±0.1a	11.2±0.8a	8.8±0.9a	3.2±0.4a	1.9±0.1a	14.6±1.8a	169.0±16.9a	134.3±16.6a	47.9±6.4a	27.0±2.6b
Lime (t ha^{-1})										
0	0.5±0.0b	5.9±0.6b	4.5±0.4c	1.6±0.2b	2.6±0.2a	6.4±0.7c	85.7±10.6c	65.2±8.3c	21.9±2.5c	37.8±3.1a
2	0.7±0.0b	8.5±0.6a	6.6±0.4b	2.4±0.2a	2.3±0.1a	10.3±0.9b	135.1±12.0b	104.5±9.1b	37.5±3.1b	36.5±3.3a
3	1.1±0.1a	12.5±0.8a	9.2±0.9a	3.2±0.4a	1.7±0.1a	16.6±1.8a	195.2±17.5a	142.8±16.3a	49.6±6.4a	27.1±2.5b
3 - Way ANOVA (F-Statistic)										
Main effects										
R	3.8*	7.5**	19.5***	9.8**	11.2**	1.4ns	2.6ns	9.7**	6.7*	10.1**
Mo	37.3***	28.1***	21.3***	15.6***	7.4**	11.2***	7.6**	7.9***	7.8***	5.7**
L	60.2***	52.0***	26.9***	16.7***	15.2***	29.0***	23.0***	14.9***	13.1***	5.0**
Interactions										
R*Mo	10.4***	7.5**	1.5ns	1.1ns	1.8ns	8.1***	6.2**	2.6ns	0.7ns	1.8ns
R*L	0.7ns	2.1ns	0.2ns	0.7ns	1.9ns	1.6ns	2.6ns	1.1ns	0.2ns	2.3ns
Mo*L	7.0***	1.9ns	1.8ns	1.7ns	0.4ns	3.3*	1.3ns	1.0ns	1.4ns	0.9ns

-R: Without *Rhizobium*; +R; With *Rhizobium*, R; *Rhizobium*, Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 2. Effect of with and without *Rhizobium*, Mo and lime supply on the macroelements uptake in shoots of *P. vulgaris* L. as measured in the glasshouse and field.

Treatments	Glasshouse experiment					Field experiment				
	P	K	Ca	Mg	S	P	K	Ca	Mg	S
			mg plant^{-1}					mg plant^{-1}		
Rhizobium										
R-	2.7±0.3b	33.3±3.1b	34.5±3.5b	8.8±0.7b	4.9±0.5b	58.6±6.6b	720.3±87.6b	722.1±79.8b	190.4±21.9b	98.1±5.9b
R+	4.2±0.2a	51.3±2.3a	64.9±4.2a	13.6±0.6a	9.3±0.5a	131.9±14.0a	1538.9±138.6a	1821.6±141.1a	413.0±38.8a	269.7±23.7a
Molybdenum (g kg^{-1})										
0	2.6±0.3b	33.5±3.2b	36.6±0.4b	8.6±0.8c	6.9±0.8a	64.8±9.4b	821.2±107.7b	893.2±129.4b	212.0±27.4b	168.3±25.9a
6	3.3±0.3b	39.6±4.0b	46.7±5.8b	10.7±1.1b	7.1±0.9a	99.1±15.9ab	1166.7±171.9ab	1287.7±182.9a	313.6±44.9ab	201.5±31.0a
12	4.6±0.3a	53.8±2.9a	65.8±5.4a	14.2±0.6a	7.3±0.6a	121.8±17.7a	1400.9±186.2a	1634.7±193.1a	379.6±52.5a	181.9±25.8a
Lime (t ha^{-1})										
0	2.9±0.3b	36.2±3.4b	39.5±4.5b	9.5±0.9b	8.3±0.9a	84.5±14.4a	1027.1±154.3a	1100.3±169.1a	277.2±45.0a	228.7±33.9a
2	3.6±0.3b	42.9±3.5ab	51.4±5.3a	11.7±1.0a	7.4±0.6a	91.9±13.9a	1077.6±139.4a	1265.6±165.1a	294.6±38.8a	183.5±25.7ab
3	4.0±0.4a	47.7±4.1a	58.3±6.6a	12.4±1.0a	5.6±0.5b	109.3±17.6a	1284.2±195.9a	1449.7±202.4a	333.4±50.4a	139.4±18.4b
3 - Way ANOVA (F-Statistic)										
Main effects										
R	28.7***	33.4***	43.0***	38.9***	46.3***	21.4***	23.4***	50.9***	23.6***	54.6***
Mo	17.3***	14.9***	13.7***	17.7***	0.2ns	4.4*	4.0*	7.7**	4.5*	0.7ns
L	5.9**	4.6*	5.6**	5.0*	6.1**	0.9ns	0.9ns	1.7ns	0.5ns	4.9*
Interactions										
R*Mo	1.0ns	3.5ns	0.6ns	2.3ns	3.9*	0.4ns	0.1ns	0.4ns	0.2ns	0.3ns
R*L	0.3ns	0.3ns	0.7ns	0.6ns	1.9ns	0.1ns	0.3ns	0.1ns	0.2ns	5.2**
Mo*L	0.9ns	0.8ns	0.6ns	0.3ns	0.2ns	0.2ns	0.2ns	0.4ns	0.1ns	0.2ns

-R: Without *Rhizobium*; +R; With *Rhizobium*, R; *Rhizobium*, Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

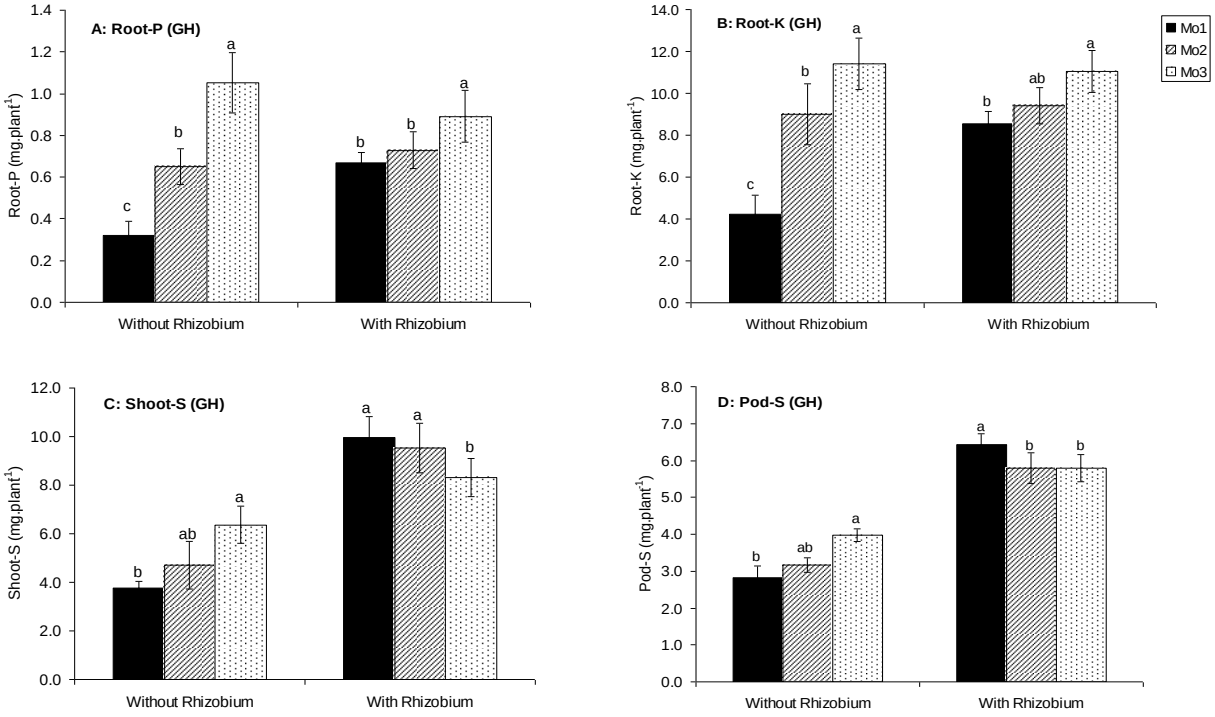

Fig1. Interactive effects of *Rhizobium* and Molybdenum on the uptake of A) Root-P; B) Root-K, C) Shoot-S and D) Pod-S of *Phaseolus vulgaris* L. grown in the glasshouse experiment. Mo1 = No Molybdenum applied, Mo2 = Molybdenum applied at 6 g kg^{-1} of seeds, Mo3 = Molybdenum applied at 12 g kg^{-1} of seeds. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$

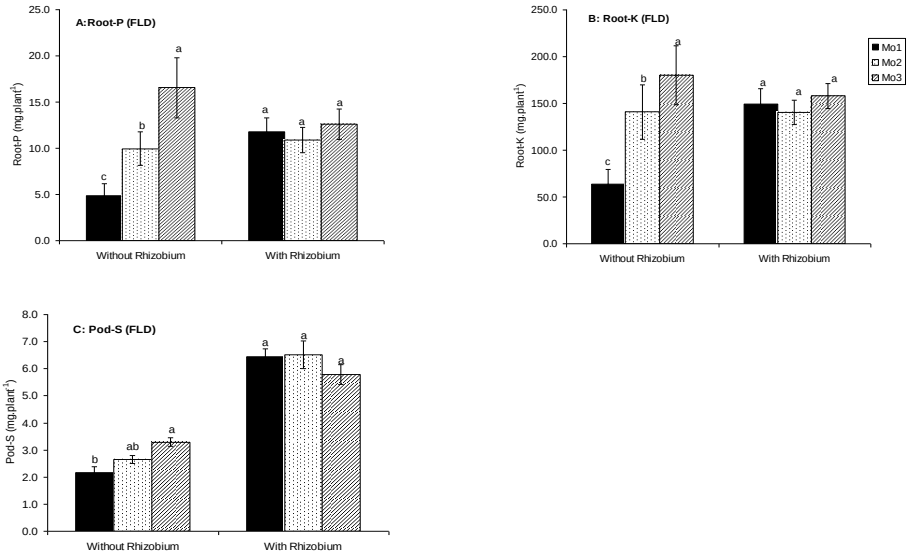

Fig 2. Interactive effects of *Rhizobium* and Molybdenum on the uptake of A) Root-P; B) Root-K and C) Pod-S of *Phaseolus vulgaris* L. grown in the field experiment. Mo1 = No Molybdenum applied, Mo2 = Molybdenum applied at 6 g kg^{-1} of seeds, Mo3 = Molybdenum applied at 12 g kg^{-1} of seeds. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$

atmospheric N_2 and change it into ammonium, a form that can be used naturally by the associated plants in different cropping systems in Africa. It is estimated that rhizobial symbiosis accounts for at least half of the annual amount of N_2 fixation in soil ecosystems (Peoples and Craswell, 1992). The productivity of acid soils is limited by two major factors, that is, phytotoxic substances such as Al of which the excessive levels may affect soil pH and induce P, Ca, Mg and Mo deficiencies (Beukes, 1995; Fageria and Baligar, 2003). *Rhizobium* inoculation and the supply of Mo and liming is one way of ameliorating such soils while increasing their fertility and productivity. In the present study, *Rhizobium* inoculation in *P. vulgaris* significantly increased the uptake of P, K, Ca, Mg and S in roots, shoots, pods and whole plant during glasshouse and field experimentation (Tables 1, 2, 3 and 4). However, greater increases over the control were observed in the uptake of Ca, S and/or Mg compared with those of P, K and/or Mg. For example, over the control, the amount of Ca and S on a whole plant basis increased by 77% and 134% in the glasshouse and field experiments respectively compared with the uptake of P, K and Mg (Table 4). Generally, macroelements uptake observed in the field was greater than in the glasshouse probably due to greater volume of soil that was available for plant roots to explore for more plant nutrients. Whether in the glasshouse or field experiment, the greater uptake of these macroelements could be ascribed to the synthesis of phytohormones, siderophores, indole 3-acetic acid (IAA) and cytokinins by the Rhizobia which directly stimulated the plant growth, thus, increasing macroelements for uptake by plant as suggested in different studies (Noel et al., 1996; Perveen et al., 2002; Khan and Zaidi, 2007; Ahmad et al., 2008; Wani et al., 2008a). Greater uptake of these macronutrients could also result from the improved rhizosphere pH as reported by Bambara and Ndakidemi (2010b). *Rhizobium* inoculation has also been reported to modify the rhizoplane by releasing dead cells which may contain macroelements or biomolecules that can solubilise unavailable to available nutrients as previously observed by Halder and Chakrabartty (1993); Abd-Alla (1994) and Chabot et al. (1996). Greater plant nutrient requirement during the N_2 fixation by legumes such as *P. vulgaris* has similarly necessitated greater uptake of such macroelements from the rhizosphere to the plant. In their field experiment, Mausumi and Raychaudhuri (2008) reported that the significant increase of P and Ca uptake in pods of groundnuts cv. JL-24 was partly attributed to *Rhizobium* inoculation. A similar result on the amount of K was also reported by Singh (1973) in pigeonpea (*Cajanus cajan* L. Millsp) during harvest. In acidic soils such as those used in the current study (Ndakidemi, 2005; Makoi, 2009; Bambara and Ndakidemi, 2010b), decreased Mo availability is attributed to weathering and excessive leaching over seasons. However, Mo in the form of MoFe protein is a key component of the most common nitrogenase enzyme and has been shown to limit N_2 fixation in cropping systems involving grain legumes (Gupta, 1992; Bellenger et al., 2008) such as *P. vulgaris*. In acid soils, Mo bioavailability is reduced by the interaction of molybdate (MoO_4^{2-}) with Fe oxides and OM (Pasricha et al., 1997). Supplying Mo will not only correct the Mo deficiency manifested in such soils, but also will improve plant growth by enhancing nodulation and N_2 fixation in *P. vulgaris*. Relative to zero control, applying Mo at 6 and 12 g kg^{-1} of seeds increased the uptake of P, K, Ca and Mg in glasshouse and field experiments (Tables 1, 2, 3 and 4) and decreased S in root organ in the field experiment (Table 1). However, greater amount of P, K, Ca and Mg was observed when Mo was supplied at 12 g kg^{-1} of seeds (Tables 1, 2, 3 and 4). For example, on a whole plant basis, the increase of these macroelements in the glasshouse were 59%, 54%, 75% and 57% over the control (i.e. Ca>P>Mg>K) whereas in the field experiment were 84%, 68%, 82% and 78% over the control (i.e. P>Ca>Mg>K) respectively (Table 4). Sulphur was however decreased by 31% under the control (Table 1). The elevated uptake of these macroelements suggests that the supplied Mo may have induced changes in the soil pH (Kaiser et al., 2005; Bambara and Ndakidemi, 2010b), thus, making these macroelements available for uptake by the plant. Increased uptake of macroelements such as K and Mg after Mo supply has been reported previously by Abd El-Samad et al. (2005). Improved availability of P, K, Ca and Mg in the study area is advantageous to cropping systems involving *P. vulgaris* which requires greater amounts of these nutrients for normal growth and N_2 fixation. Although S was only significant in root organ, it was decreased when Mo was supplied at 12 g kg^{-1} of seeds. The decrease in the uptake of S was probably due to the sulfate anion (SO_4^{2-}) behaving more like the molybdate anion (MoO_4^{2}). As these anions use the same transport system may have acted antagonistically just as reported by Sims et al. (1979) and Marschner (1995). Amelioration of acid soils with lime provides direct availability of Ca and Mg for uptake by plant, better conditions for growth of bacterial cells, change in soil pH and increased uptake of P and Mo. Compared with the zero control, applying lime at 2 t ha^{-1} and 3 t ha^{-1} significantly increased the uptake of P, K, Ca and Mg (Tables 1, 2, 3 and 4) and decreased S uptake in shoot and whole plant (Table 2 and 4) in the glasshouse experiment. Generally, greater uptake of these macroelements was observed when lime was supplied at 3 t ha^{-1}. For example, on a whole plant basis, P, K, Ca and Mg was significantly increased by 48%, 42%, 53% and 39% respectively over the control in decreasing order as Ca>P>K>Mg (Table 4). Generally, similar trend was observed in shoots and pods (Tables 2 and 3) and to a lesser extent in roots (Table 1). The increased uptake of these macroelements was attributed to improved soil pH as similarly reported by Mora and Barrow (1996); Andrade et al. (2002) and Bambara and Ndakidemi (2010b). Increased soil P, K, Ca and Mg by liming has similarly been reported by Jansen van Rensburg et al. (2010). The mechanism involved is that, under acidic conditions Ca and Mg have low exchange power over the acid causing ions such as H^+ in the soil solution. But as lime is supplied, OH^- ions are added into the soil solution, thus, increasing the exchange power for the polyvalent cations such as Ca^{2+} and Mg^{2+} and making them available for plant uptake (Marschner, 1989). Likewise, for high uptake rate of these macroelements, high concentration of polyvalent cations including Ca^{2+} and Mg^{2+} should be maintained along the apoplasmic pathway which is necessary for loading of these macroelements in the roots apoplast. For this to happen, increased external pH is required. In this study, the overall effects of lime on the soils include among others, increased soil pH, Ca and Mg saturation, neutralization of phytotoxic concentrations, increase in P availability and improved nutrient uptake by plants (Nicholaides et al., 1983; Oguntoyinbo et al., 1996). Secondly, as liming application in acid soils improves the soil pH and the uptake of P, Ca, Mg and K in *P. vulgaris*, the adsorption of *Rhizobium* at the root surface and nodulation will be enhanced along with the ability of root exudates to induce the expression of nodulation genes (Richardson et al. 1988; Caetano-Anolles et al., 1989). This positive change is of great contribution to the acid soils and their productivity when used for different cropping systems. In this study, remarkable differences were

Table 3. Effect of with and without *Rhizobium*, Mo and lime supply on the macroelements uptake in pods of *P. vulgaris* L. as measured in the glasshouse and field.

Treatments	Glasshouse experiment					Field experiment				
	P	K	Ca	Mg	S	P	K	Ca	Mg	S
			mg plant^{-1}					mg plant^{-1}		
Rhizobium										
R-	5.4±0.4b	40.5±2.7b	11.0±0.7b	5.2±0.3b	3.3±0.2b	4.4±0.3b	33.6±2.5b	9.1±0.7b	4.3±0.3b	2.7±0.1b
R+	9.4±0.5a	66.3±2.8a	19.9±1.0a	9.3±0.4a	6.0±0.2a	9.8±0.5a	68.9±2.7a	20.8±1.0a	9.7±0.4a	6.2±0.2a
Molybdenum (g kg^{-1})										
0	6.1±0.6b	44.7±4.2b	12.5±1.1c	6.3±0.6b	4.6±0.4a	5.7±0.7c	41.7±4.7c	11.8±1.3c	5.9±0.7b	4.3±0.5a
6	7.0±0.6b	50.2±3.8b	14.4±1.2b	6.9±0.6b	4.5±0.4a	7.1±0.8b	51.6±5.1b	15.1±1.6b	7.1±0.8a	4.6±0.5a
12	9.1±0.7a	65.3±3.8a	19.4±1.5a	8.6±0.6a	4.9±0.3a	8.3±0.7a	60.5±4.0a	18.0±1.5a	8.1±0.6a	4.5±0.3a
Lime (t ha^{-1})										
0	5.9±0.5b	43.7±3.4b	12.2±1.0c	6.1±0.5b	4.9±0.4a	5.7±0.6c	42.2±4.3c	12.0±1.4c	6.0±0.7b	4.7±0.5a
2	7.6±0.6a	55.1±4.0a	15.8±1.2b	7.5±0.6a	4.8±0.4a	7.1±0.7b	51.5±4.8b	14.9±1.5b	7.0±0.7b	4.5±0.4a
3	8.6±0.8a	61.5±4.6a	18.3±1.6a	8.3±0.7a	4.3±0.3a	8.4±0.8a	60.1±4.8a	18.0±1.7a	8.1±0.7a	4.2±0.3a
3 - Way ANOVA (F-Statistic)										
Main effects										
R	71.5***	80.1***	138.1***	98.8***	111.5***	142.7***	146.3***	170.9***	147.8***	184.7***
Mo	13.4***	18.3***	29.0***	11.9***	0.9ns	11.3***	14.0***	16.2***	7.9**	0.4ns
L	10.8***	13.1***	21.7***	10.3***	2.1ns	12.0***	12.5***	14.9***	7.9***	1.2ns
Interactions										
R*Mo	0.1ns	1.1ns	0.0ns	0.7ns	4.2*	0.9ns	1.6ns	0.7ns	1.2ns	4.3*
R*L	0.7ns	0.8ns	1.2ns	0.9ns	1.3ns	0.6ns	0.3ns	0.6ns	0.3ns	1.4ns
Mo*L	1.5ns	1.4ns	3.3*	1.7ns	0.8ns	0.6ns	0.3ns	0.6ns	0.4ns	0.4ns

-R: Without *Rhizobium*; +R; With *Rhizobium,* R; *Rhizobium,* Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 4. Effect of with and without *Rhizobium*, Mo and lime supply on the macroelements uptake in whole plant in *P. vulgaris* L. as measured in the glasshouse and field.

Treatments	Glasshouse experiment					Field experiment				
	P	K	Ca	Mg	S	P	K	Ca	Mg	S
			mg plant^{-1}					mg plant^{-1}		
Rhizobium										
R-	8.8±0.6b	82.0±5.9b	51.1±4.5b	16.0±1.1b	10.2±0.5b	73.4±7.5b	882.1±96.5b	817.3±83.7b	225.4±23.0b	129.8±6.7b
R+	14.4±0.7a	127.3±5.0a	92.7±5.2a	25.6±1.1a	17.7±0.7a	153.5±14.3a	1757.0±139.3a	1964.7±142.4a	464.8±38.5a	314.5±24.2a
Molybdenum (g kg^{-1})										
0	9.2±0.9c	84.6±7.6c	53.7±5.4c	16.6±1.5c	13.9±1.3a	78.9±10.5b	969.2±118.6b	983.3±136.2c	244.6±29.8b	211.9±27.7a
6	10.9±0.9b	99.0±7.4b	67.8±7.0b	19.9±1.6b	14.0±1.2a	116.6±16.5ab	1359.0±177.4ab	1402.8±187.0b	355.1±45.7a	241.0±32.0a
12	14.6±0.9a	130.3±6.5a	94.1±7.0a	26.0±1.4a	14.1±0.8a	144.8±18.1a	1630.4±188.0a	1787.0±193.8a	435.5±51.9a	213.5±27.1a
Lime (t ha^{-1})										
0	9.2±0.7c	85.8±6.6c	56.1±5.6c	17.1±1.3c	15.8±1.3a	96.6±15.2a	1155.0±161.7a	1177.5±174.2a	305.0±46.6a	271.2±35.3a
2	11.9±0.9b	106.5±7.3b	73.7±6.6b	21.5±1.5b	14.4±1.0a	109.4±14.6a	1264.1±146.0a	1385.0±170.5a	339.1±40.0a	224.6±27.0ab
3	13.7±1.1a	121.7±8.8a	85.7±8.3a	23.9±1.9a	11.7±0.8b	134.3±18.1a	1539.5±199.0a	1610.5±204.1a	391.2±50.4a	170.6±18.7b
3 - Way ANOVA (F-Statistic)										
Main effects										
R	74.5***	76.9***	68.7***	81.8***	84.9***	25.1***	27.0***	57.3***	29.1***	63.7***
Mo	23.8***	27.4***	22.2***	27.4***	0.0ns	5.7**	5.2**	9.4**	6.2**	0.7ns
L	15.6***	16.3***	11.8***	13.8***	9.1***	1.9ns	1.8ns	2.7ns	1.3ns	6.3**
Interactions										
R*Mo	0.7ns	3.8*	0.7ns	1.6ns	5.6**	0.2ns	0.0ns	0.3ns	0.2ns	0.4ns
R*L	0.6ns	0.5ns	1.0ns	1.0ns	2.6ns	0.2ns	0.4ns	0.2ns	0.2ns	6.2**
Mo*L	1.8ns	1.4ns	1.3ns	1.1ns	0.2ns	0.3ns	0.3ns	0.5ns	0.2ns	0.2ns

-R: Without *Rhizobium*; +R; With *Rhizobium,* R; *Rhizobium,* Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

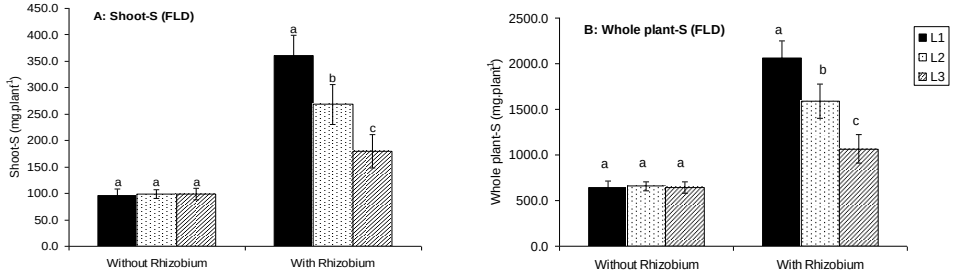

Fig 3. Interactive effects of *Rhizobium* and Lime on the uptake of A) Shoot-S, and B) Whole plant-S of *Phaseolus vulgaris* L grown in the field experiment. L1 = No lime applied, L2 = Lime applied at 2 t ha^{-1}, L3 = Lime applied at 3 t ha^{-1}. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$**.**

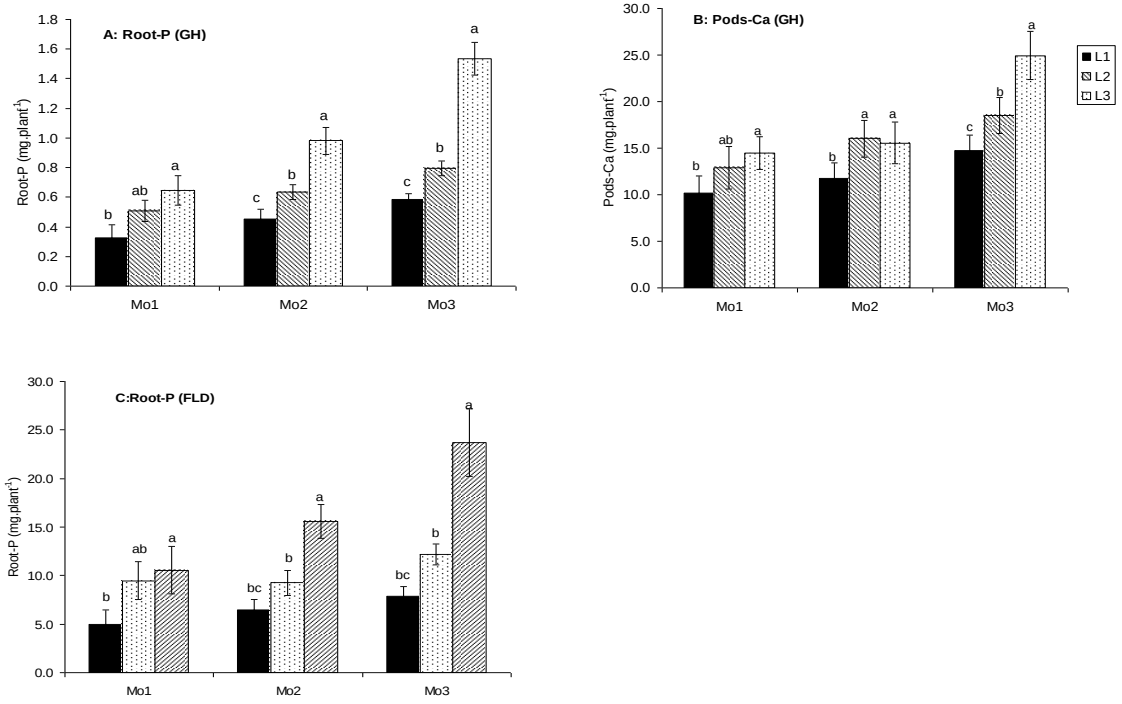

Fig 4. Interactive effects of Molybdenum and Lime on A) Root-P and B) Pods-Ca of *Phaseolus vulgaris* L. grown in the glasshouse and C) Root-P grown in the field experiments. Mo1 = No Molybdenum applied, Mo2 = Molybdenum applied at 6 g kg^{-1} of seeds, Mo3 = Molybdenum applied at 12 g kg^{-1} of seeds. L1 = No lime applied, L2 = Lime applied at 2 t ha^{-1}, L3 = Lime applied at 3 t ha^{-1}. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$.

observed between field and glasshouse experiments on the uptake of macroelements in all plant organs. These differences may be attributed to large volume of soil exposed to the roots of the *P. vulgaris* to search for nutrients in the field compared with that of glasshouse experiment. Exposure of plant roots to large volume of soil will enable the plant to scavenge for more water and macronutrients. Significant interaction between *Rhizobium* x Mo, *Rhizobium* x lime and Mo x lime was observed in glasshouse and field experiments. For example, the interaction between *Rhizobium* x Mo significantly increased the amount of P and K in the roots of *P. vulgaris* regardless of adding or not adding *Rhizobium* inoculants (Figs. 1A and B). However, distinct uptake of P and K was observed under no *Rhizobium* inoculation and their amount increased as Mo was increased to 6 g kg^{-1} of seeds compared with where *Rhizobium* inoculation was applied. The increased uptake of these macroelements in the roots was probably attributed to the supply of Mo which may have raised the soil pH (Oguntoyinbo et al., 1996; Bambara and Ndakidemi, 2010b), thus, making them available for plant uptake. It was also previously reported by Ssali and Keya (1983) and Bergersen (1991) that N_2 fixation is a very expensive process and requires greater amounts of nutrients including P, K and Mo. This argument could probably explain why the uptake of these macroelements was not as distinct with *Rhizobium* inoculated treatments compared with no *Rhizobium* inoculation, suggesting that greater amounts of P, K and Mo may have been utilized during N_2 fixation process and nodulation compared with no *Rhizobium* inoculation. Combining with or without *Rhizobium* with Mo significantly ($P \leq 0.05$) affected the uptake of S in shoots and pods in both glasshouse and field studies (Figs. 1C, D and 2C). Regardless of the Mo applied, the amount of S was significantly higher with *Rhizobium* inoculation compared with no *Rhizobium* inoculation. The result showed that by increasing supply of Mo under no rhizobia inoculation, the uptake of S in the shoots and pods was increased, and, significant amount was observed when Mo was applied at 6 g kg^{-1} of seeds (Figs. 1C, D and 2C). On the contrary, with *Rhizobium* inoculation, the uptake of S showed a general declining trend suggesting that the uptake of S in shoots and pods was negatively affected by the supply of Mo, more so at 6 g kg^{-1} of seeds. There was significant ($P \leq 0.05$) *Rhizobium* x lime interaction on the uptake of S in shoots and whole plant of *P. vulgaris* grown in the field experiments (Fig. 3A and B). Generally, the uptake of S in shoots and whole plant without *Rhizobium* inoculation did not change regardless of the amount of lime supplied. Although *Rhizobium* inoculation improved amount of S relative to no *Rhizobium* inoculation, the uptake of S was significantly ($P \leq 0.05$) reduced, with the lowest uptake observed when lime was supplied at 3 t ha^{-1}, thus, suggesting that lime application has a negative effect on the uptake of S in shoot and whole plant. The data has also showed significant ($P \leq 0.05$) Mo x lime interaction in the uptake of P in roots and Ca in pods (Fig. 4A, B and C). From this data, combining Mo and lime increased the uptake of P and Ca. Greater uptake of these macroelements was observed when Mo at 6 g kg^{-1} of seeds was combined with lime at 3 t ha^{-1} and was ascribed to the change in soil pH caused by both Mo and lime (Chang and Sung, 2004; Karaivazoglou et al., 2007), which may have made the nutrients to be more available for plant uptake. This data suggest that in cropping systems involving *P. vulgaris* growing in acid soils, greater uptake of P and Ca can be achieved when Mo at 6 g kg^{-1} of seeds is combined with lime at 3 t ha^{-1}.

Materials and methods

Site location and description

The experiments were conducted in the glasshouse of the Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht from August 2008 to January 2009 while the field study was conducted at the Agricultural Research Council (ARC) 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 rainfall on the farm is 713.4 mm and the means annual day and night temperatures were 22.6°C and 11°C respectively. The experimental site had a previous history of grape cultivation. The soil type was skeletic leptosol according to FAO classification (FAO, 2001). Following land preparation, but prior to planting, soil samples at 0 - 20 cm depth were collected from the experimental plots in a zigzag mode, pooled, and sub-samples taken for chemical analysis.

Field experimental design

The experimental treatments (in both greenhouse and field) consisted of 2 levels of *Rhizobium* inoculation (with *Rhizobium* and without *Rhizobium*), 3 levels of dolomitic agricultural lime (0, 2 and 3 t ha^{-1}) and 3 levels of molybdenum (0, 6 and 12 g kg^{-1} of seeds). The experimental layout was 3-factorial in a randomised complete block design. Four replicates were used per treatment. In the field, experimental plots measured 4 m × 4 m (16 m^2) with 4 rows spaced 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 populations were pegged at 200,000 plants ha^{-1}. Planting was done after ploughing, harrowing, and lime application was done 2 weeks before planting. For the glasshouse experiment, enough soil was collected from the ploughed land of the field experimental site. Four kg of soil was then packed in 4 kg pot size and two seeds were sown in each pot. Twelve hours before planting, seeds were soaked into molybdenum solution. The control was also soaked in a water solution containing zero 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 registrasie nr. L1795 wet 36/1947) in the planting hole. The inoculants used were obtained from the University of Pretoria, South Africa. In the greenhouse, 200 mLs of water was supplied in each pot per day. In the field experiment, irrigation water was supplied through sprinklers for 15 minutes per day. After germination, the seedlings were thinned to one in both glasshouse and field experiments.

Plant harvest and sample preparation

At 60 DAP, 10 common bean plants were sampled from the middle rows of each plot. The plants were carefully dug out with their entire root system, washed and divided into roots, shoots, pods. The plant parts were oven-dried at 60°C for 48 h, weighed, ground into a fine powder (0.85 mm) and stored prior to the tissue nutrient analysis. In the glasshouse, plants were sampled at 60 DAP as explained above.

Measurement of macroelements in plant tissue

Measurements of P, K, Ca, Mg and S were determined based on Giron (1973) and FSSA (1974). Nutrient uptake (mg plant^{-1}) was then calculated as the product of nutrient concentration (mg g^{-1}, data not shown) and the weight of the plant part dry matter (g plant^{-1}).

Statistical analysis

A 3-Way ANOVA was used to analyse the macroelements uptake in *P. vulgaris*. Analysis of data was performed using STATISTICA program 2010 (StatSoft Inc., Tulsa, OK, USA). Where the f-value was found to be significant, Fisher's least significant difference was used to compare treatment means at P=0.05 (Steel and Torrie, 1980).

Conclusion

This study shows that *Rhizobium* inoculation and the supply of Mo and lime can be of great benefit to farmers growing grain legumes such as *P. vulgaris* in acid soils. Additionally, these inputs have showed to improve the uptake of macroelements such as P, K, Ca and Mg, which are greatly required by plant for growth, nodulation and N_2 fixation.

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