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Rhizosphere phosphatase enzyme activities and secondary metabolites in plants as affected by the supply of *Rhizobium*, lime and molybdenum in *Phaseolus vulgaris* L.

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Abstract

Knowledge about changes of enzyme activities in the rhizosphere soil and secondary metabolites in *Phaseolus vulgaris* L. plant is necessary to understand especially during the management of acid soils. A glasshouse and field experiments were therefore conducted to investigate the effects of applying or not applying *Rhizobium* inoculation, molybdenum (0, 6, 12 g kg^{-1} seed) and lime (0, 2, 3 t ha^{-1}) on the rhizosphere enzymes activities, flavonoids and anthocyanins concentration in *P. vulgaris* L. Glasshouse and field experiments indicated that *Rhizobium* inoculation, Mo at 12 g kg^{-1} seed and lime at 3 t ha^{-1} supply significantly showed lowest acid and alkaline phosphatase activities and flavonoids and anthocyanins concentration compared with control treatments which showed highest values. The lower rhizosphere activities of these enzymes and plants' secondary metabolites with *Rhizobium* and greater supply of Mo and lime suggest less stress for mineral elements essential for plant growth such as N, Ca, and Mo compared with control. Furthermore, the data suggest that the lower the stress for mineral elements in the rhizosphere soils of legumes such as *P. vulgaris* L. the lower the phosphatase activities and plants' flavonoids and anthocyanins concentrations.

Keywords: Acid phosphatase, alkaline phosphatase, anthocyanins, common bean flavonoids, nutrient concentration, rhizosphere soil

Abbreviations: Al-Aluminium; ANOVA-Analysis of variance; Ca-Calcium; DAP-Days after planting; Fe-Iron; IAA- Indole acetic acid; K-Potassium; Mg-Magnesium; Mo – Molybdenum; N-Nitrogen; OA-Organic acid; OM-Organic matter; Pi-Inorganic phosphorous; Po-Organic phosphorous; (R+)-With *Rhizobium*; (R-)-Without *Rhizobium*; Zn-Zinc

Introduction

Soil enzyme activities in the rhizosphere soils of legumes serve many important roles. They include release of soil organic (P_o) into inorganic (P_i) phosphorous (Speir and Ross, 1978; Tabatabai, 1994; Makoi and Ndakidemi, 2008; Makoi et al., 2010a), cycling of nutrients, fertilizer use efficiency, soil microbiological activities and act as indicators of soil fertility change (Dick et al., 2000; Ndakidemi, 2005 and 2006). These rhizosphere enzyme activities are similarly known to serve as an indicator of soil fertility, a catalyst for soil functions such as organic matter (OM) decomposition, N_2 fixation, nitrification, and denitrification (Dick, 1997; Nadiya et al., 2000; Makoi and Ndakidemi, 2008). They are produced when plants and soil micro organisms are subjected to nutrient stress such as P (Ndakidemi, 2005 and 2006; Makoi and Ndakidemi, 2008), calcium (Ca) (Speir and Ross, 1978; Bremner and Mulvaney, 1978) and molybdenum (Mo) (Sugiura et al., 1981; Gellatly et al., 1994; Guo et al., 1998; Bozzo et al., 2002; Lopez et al., 2007). On the other hand, secondary metabolites such as flavonoids and anthocyanins are diverse group of phytochemicals that are produced by various plants in high quantities (Dixon and Steele, 1999). They exhibit a wide range of biological activities mainly from their antioxidant properties and ability to modulate several enzymes or cell receptors (Hodek et al., 2002). Flavonoids for example, play an important role in plant growth and development, defence of plants against insect pests and diseases (Dixon and Harrison, 1990; Dixon and Steele, 1999; Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007; Makoi et al., 2010b).

The common bean (*P. vulgaris* L.) is a major grain legume grown and consumed in Africa and the world in general. However, yields *P. vulgaris* L. in Africa are very low with only 650 $kg.ha^{-1}$ (Mukoko et al., 1995; Singh, 1999; Mloza-Banda et al., 2003). The poor yields are partly due to infertility caused by acidic soils which have low nutrient contents including Ca^{2+} (Wortman et al., 1995 and1998; Lunze et al., 2007), Mo content (Liebenberg, 2002) and compatible *Rhizobium* for adequate N_2 fixation. Supplying these mineral elements may not only improve the mineral element concentration in such soils, but will also improve plant growth and grain yield from *P. vulgaris* L. Although some studies on the effect of such treatments on some physiological attributes are available (Bambara and Ndakidemi, 2010a, b, c), the effect of *Rhizobium* inoculation

and the supply of Mo and lime on soil enzyme activities and plants' secondary metabolites are limited. This study assesses the effects of *Rhizobium*, molybdenum and lime supply in *P. vulgaris* L. on the rhizosphere phosphatase enzyme activities and plants' secondary metabolites such as flavonoids and anthocyanins.

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 experiment was conducted 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 rainfall on the farm is 713.4 mm and 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 sandy loam (Glenrosa, Hutton form) according to the soil classification working group (SCWG, 1991), which is equivalent to skeletic leptosol according to FAO classification (FAO, 2001). Following land preparation, but prior to planting, soil samples were collected for nutrients analysis. 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. Before planting the soil characteristics of the field plots have previously been reported (Bambara and Ndakidemi, 2010b; Makoi et al., 2010a and b).

Field experimental design

The experimental treatments consisted of 2 levels of *Rhizobium* inoculation (with *Rhizobium* and without *Rhizobium*), 3 levels of 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, and field plots measured 4 m × 4 m (16 m^2) with 4 rows spaced at 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. 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.

Collection and preparation of rhizosphere soil

At 60 d after planting (DAP), rhizosphere soil was collected from around the roots of *P. vulgaris* L. plants for the bioassay of acid and alkaline phosphatase activities. Soil was collected by carefully digging up each plant with its roots intact. The loose soil around the roots was shaken off and the soil adhering to the roots (here after referred to as rhizosphere soil) was collected into a pre-labelled plastic bag. About 50 g of soil was collected from 5 - 10 plants $plot^{-1}$. The rhizosphere soil samples were taken to the laboratory and frozen prior to bioassay for phosphatase activity.

Bioassay of acid and alkaline phosphatase activity in rhizosphere soil

The activity of acid and alkaline phosphatase activities in the rhizosphere were assayed as described in Makoi et al. (2010a). The *p*-nitrophenyl phosphate tetrahydrate was used in the colorimetric assay of acid and alkaline phosphatases. One mL *p*-nitrophenyl phosphate tetrahydrate was dissolved in acetate buffer previously adjusted to pH 6.5 with 0.1 M HCl and to pH 11.0 with 0.1 M NaOH for acid and alkaline phosphatase, respectively. For each enzyme activity, 1.0 g of fresh rhizosphere soil in duplicates was transferred to a 50 mL Erlenmeyer flask and each treated separately with 0.2 mL of toluene and 4 mL of modified universal buffer (MUB) at pH 6.5 or 11 for acid or alkaline phosphatases respectively. For each soil sample, controls were included where *p*-nitrophenyl phosphate tetrahydrate was added after halting the reaction by adding 1 mL of 0.5 M NaOH and 4 mL of 0.5 M $CaCl_2$ immediately before filtration. Samples were mixed thoroughly and incubated at 37ºC for 1h. Following incubation, enzyme activity was halted by addition of 1 mL of 0.5 M NaOH and 4 mL of 0.5 M $CaCl_2$. The contents were mixed and filtered through Whatman No. 2 filter paper. The supernatant was transferred to vials and the absorbance of the supernatant read at 420 nm using a spectrophotometer (UV Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E). In order to account for non-enzymatic substrate hydrolysis, values for controls were subtracted from sample replicates. After correction for soil moisture content, the enzyme activity was expressed on soil dry wt basis as µg *p*-$nitrophenol.g^{-1}$ soil dry weight h^{-1}. One unit of acid phosphatases activity was defined as the activity per gram soil which produced 1 µmol *p*-nitrophenol h^{-1}.

Plant harvest and sample preparation

At 60 d after planting, 10 common bean (*P. vulgaris* L.) 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 and 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 bioassay for flavonoids and anthocyanins concentrations.

Bioassay of levels of flavonoids and anthocyanins in the shoots parts of P. vulgaris L.

Flavonoids and anthocyanins concentration in shoots of *P. vulgaris* L. were bioassay as described in Makoi et al. (2010b). In this method, 0.10 g of well-ground (0.85 mm) shoot material was weighed and mixed with 10 mLs of acidified methanol prepared from a ratio of 79:20:1 MeOH H_2O HCl. The mixture was incubated for 72 h in darkness for auto-extraction, filtered through Whatman paper Number 2 and absorbance of the clear supernatant measured spectrometrically at 300, 530, and 657 nm using acidified methanol as standard. Concentrations of

Table 1. Effect of with and without *Rhizobium*, Mo and lime supply on acid and alkaline phosphatises (µg p-nitrophenol.g^{-1} soil dry weight.h^{-1}) in the rhizosphere soils of *P. vulgaris* L. as measured in field and in glasshouse.

Treatments	Acid phosphatases		Alkaline phosphatases	
	Field	Glass	Field	Glass
Rhizobium				
R-	0.178±0.014a	0.180±0.013a	0.069±0.005a	0.071±0.005a
R+	0.138±0.007b	0.144±0.007b	0.056±0.003b	0.059±0.003b
Molybdenum ($g.kg^{-1}$)				
0	0.202±0.019a	0.199±0.019a	0.077±0.007a	0.080±0.007a
6	0.139±0.007b	0.153±0.008b	0.060±0.003b	0.062±0.003b
12	0.134±0.007b	0.134±0.007b	0.051±0.003b	0.053±0.003b
Lime ($t.ha^{-1}$)				
0	0.182±0.015a	0.195±0.014a	0.078±0.005a	0.079±0.006a
2	0.157±0.012ab	0.159±0.011b	0.062±0.004b	0.064±0.004b
3	0.135±0.012b	0.132±0.012b	0.049±0.004c	0.051±0.005b
3 - Way ANOVA (F-Statistic)				
Rhizobium	8.37**	7.26**	7.912**	5.54*
Molybdenum	10.27***	8.44***	10.43***	9.11***
Lime	3.95*	7.34**	12.43***	9.63***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; according to Fischer least significance difference. (SE = Standard error).

flavonoids were measured at 300 nm and expressed as Abs g.DM^{-1} (Mirecki and Teramura, 1984), while anthocyanin concentration in seed exudates was measured as Abs_{530} - 1/3Abs_{657} (Lindoo and Caldwell, 1978) and expressed as Abs g DM^{-1}

Statistical analysis

A 3-factorial design (3-Way ANOVA) was used to analyse rhizosphere acid and alkaline phosphatase activities, as well as flavonoids and anthocyanins concentration in shoots of *P. vulgaris* L. Analysis of data was performed using STATISTICA program 2009 (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).

Results

Effects of Rhizobium, molybdenum and lime on acid phosphatase activities in the rhizosphere soil of P. vulgaris L.

There were significant ($P \leq 0.05$) differences in acid phosphatase activity in the rhizosphere of *P. vulgaris* L. associated with *Rhizobium* inoculation, Mo and lime supply in glasshouse and field experiments (Table 1). For example, *Rhizobium* inoculation decreased the levels of acid phosphatase activity in the rhizosphere soil by 22.2% (field) and 20.0% (glasshouse) compared with the uninoculated control. Supplying Mo at 6 and 12 g kg^{-1} of seeds during field experiment decreased ($P \leq 0.05$) the acid phosphatase activity by 31.3% and 33.6% respectively compared with the control. Similarly, this activity was decreased by 23.2% and 32.9% in glasshouse experiment (Table 1). Furthermore, lime at 2 and 3 t ha^{-1} significantly ($P \leq 0.05$) decreased the levels of acid phosphatase activity by 14.0% and 25.7% in field experiment while in glasshouse these levels were reduced by 18.2% and 32.1% respectively compared with the control treatment (Table 1).

Effects of Rhizobium, molybdenum and lime on alkaline phosphatase activities in the rhizosphere soil of P. vulgaris L.

There were significant ($P \leq 0.05$) differences in alkaline phosphatase activity in the rhizosphere of *P. vulgaris* L. associated with *Rhizobium* inoculation, Mo and lime supply in glasshouse and field experiments (Table 1). For example, *Rhizobium* inoculation decreased the levels of alkaline phosphatase activity in the rhizosphere soil by 19.3% (field) and 17.3% (glasshouse) compared with the uninoculated control. Supplying Mo at 6 and 12 g kg^{-1} of seeds during field experiment decreased ($P \leq 0.05$) the alkaline phosphatase activity by 22.5% and 33.8% respectively compared with the control. Similarly, this activity was decreased by 22.4% and 33.7% in glasshouse experiment (Table 1). Furthermore, lime at 2 and 3 t ha^{-1} significantly ($P \leq 0.05$) decreased the levels of alkaline phosphatase activity by 20.5% and 37.3% in field experiment while in glasshouse these levels were reduced by 19.4% and 35.4% respectively compared with the control treatment (Table 1).

Table 2. Effect of with and without *Rhizobium*, Mo and lime supply on the secondary metabolites (Abs.g DM^{-1}) in shoots of *P. vulgaris* L. as measured in field and in glasshouse.

Treatments	Flavonoids		Anthocyanins	
	Field	Glass	Field	Glass
Rhizobium				
R-	0.210±0.005a	0.206±0.005a	0.036±0.002a	0.033±0.002a
R+	0.174±0.009b	0.172±0.008b	0.030±0.002b	0.032±0.002a
Molybdenum ($g.kg^{-1}$)				
0	0.222±0.006a	0.223±0.005a	0.040±0.002a	0.039±0.002a
6	0.191±0.008b	0.192±0.006b	0.033±0.002b	0.033±0.002b
12	0.162±0.009c	0.153±0.009c	0.027±0.002c	0.027±0.002c
Lime ($t.ha^{-1}$)				
0	0.222±0.007a	0.210±0.007a	0.043±0.002a	0.042±0.002a
2	0.192±0.008b	0.190±0.008b	0.032±0.001b	0.032±0.001b
3	0.162±0.009c	0.167±0.010c	0.024±0.001c	0.024±0.001c
3 - Way ANOVA (F-Statistic)				
Rhizobium	44.39***	47.38***	34.603***	1.252ns
Molybdenum	43.22***	67.41***	44.183***	25.982***
Lime	42.19***	25.53***	94.004***	58.512***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at ***: $P \leq 0.001$ according to Fischer least significance difference. (ns = not significant, SE = Standard error).

Effects of Rhizobium, molybdenum and lime on flavonoids concentrations in the shoots of Phaseolus vulgaris L.

The flavonoids concentration (Abs g DM^{-1}) differed ($P \leq 0.05$) with *Rhizobium* inoculation, Mo and lime supply (Table 2). The data showed that with *Rhizobium* inoculation, levels of flavonoids in shoots of *P. vulgaris* L. decreased by 16.8% (field), 16.5% (glasshouse) relative to control (Table 2). Supplying Mo at 6 and 12 g kg^{-1} of seeds in field experiment decreased ($P \leq 0.05$) the levels of flavonoids by 14.0% and 27.0% respectively compared with the control. Similarly, this concentration was decreased by 22.4% and 33.7% in glasshouse experiment (Table 2). Furthermore, lime at 2 and 3 t ha^{-1} significantly ($P \leq 0.05$) decreased the levels of flavonoids by 13.1% and 26.8% in field experiment while in glasshouse these levels were reduced by 9.3% and 20.5% respectively compared with the control treatment (Table 2).

Effects of Rhizobium, molybdenum and lime on anthocyanin concentrations in the shoots of Phaseolus vulgaris L.

The anthocyanin concentration (Abs g DM^{-1}) differed ($P \leq 0.05$) with *Rhizobium* inoculation, Mo and lime supply (Table 2). The data showed that with *Rhizobium* inoculation, levels of anthocyanin in shoots of *P. vulgaris* L. decreased by 17.5% (field), 4.5% (glasshouse) relative to control (Table 1). Supplying Mo at 6 and 12 g kg^{-1} of seeds during field experiment decreased ($P \leq 0.05$) the anthocyanin concentration by 17.1% and 31.5% respectively compared with the control. Similarly, this activity was decreased by 24.0% and 42.6% in glasshouse experiment (Table 2). Furthermore, lime at 2 and 3 $t.ha^{-1}$ significantly ($P \leq 0.05$) decreased the levels of anthocyanins by 15.4% and 30.9% in field experiment while in glasshouse these levels were reduced by 23.6% and 42.7% respectively compared with the control treatment (Table 2).

Interactive effect of Rhizobium, molybdenum on the concentration of flavonoids in shoots of P. vulgaris L.

There was significant ($P \leq 0.05$) interaction between *Rhizobium*, molybdenum and lime in flavonoids concentration in *P. vulgaris* L. shoots (Figs. 1, and 2). Whether grown in the glasshouse or field, combining *Rhizobium* inoculation with Mo lowered the levels of flavonoids concentration compared with no *Rhizobium* inoculation (Fig. 1A, B). Although the data from glasshouse experiment interaction showed no significant difference when *Rhizobium* inoculation was combined with lime, in the field however, *Rhizobium* inoculation combined with lime significantly reduced the levels of flavonoids concentration compared with when *Rhizobium* was not combined with lime (Fig. 2).

Discussion

In this study, acid and alkaline phosphatase activities in the rhizosphere soils as well as flavonoids and anthocyanins in shoots of *P. vulgaris* L. were assayed with the objective of quantifying their levels when supplied with or without *Rhizobium*, Mo and lime. The data showed significant differences in acid and alkaline phosphatase activities with the supply of *Rhizobium*, Mo and lime showing lowest levels with

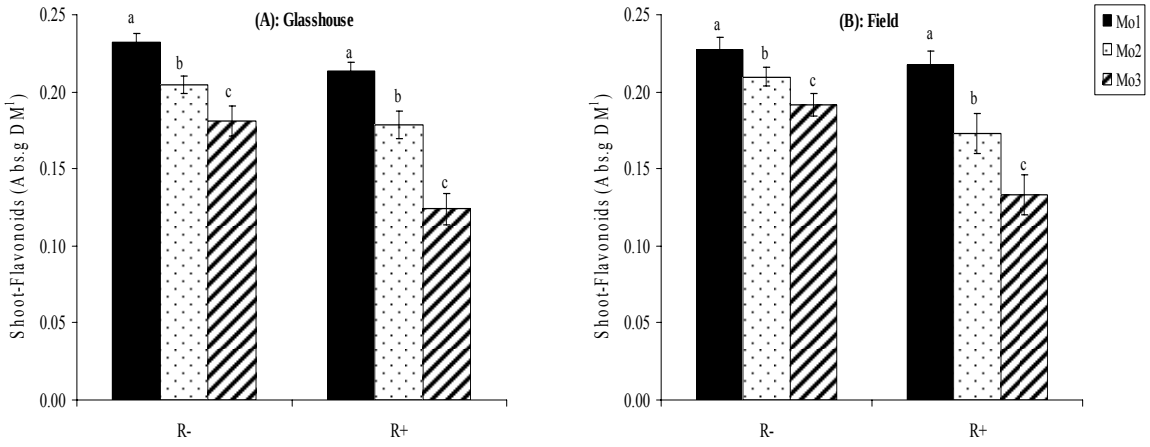

Fig. 1: Interactive effects of *Rhizobium* and molybdenum on shoot flavonoids concentration A) Glasshouse and B) Field experiments (R-: Without *Rhizobium*, R+: With *Rhizobium*, Mo1: molybdenum at 0 g.kg^{-1} seed, Mo2: molybdenum at 6 g.kg^{-1} seed and Mo3: molybdenum at 12 g.kg^{-1} seed)

addition of these with supplies (Table 1). The rhizosphere soil of *P. vulgaris* L. supplied with Mo at 12 g kg^{-1} seeds and lime at 3 t ha^{-1} secreted less acid and alkaline phosphatases compared with the other treatments (i.e. 0 and 6 g kg^{-1} seeds for Mo as well as 0 and 2 t ha^{-1} for lime) during glasshouse and field experiments (Table 1). The lower acid and alkaline phosphatase activities exhibited in the rhizosphere of *P. vulgaris* L. suggest less stress for mineral elements for plant growth. For instance, supply of *Rhizobium* to *P. vulgaris* L. showed lower levels of acid and alkaline phosphatases in the rhizosphere soil. The lower levels of these compounds reflected how the micro-organisms in the rhizosphere reacts and interacts to the many metabolites released by the roots of grain legumes such as *P. vulgaris* L. in a variety of ways including N_2 fixation, enhanced solubilization of P, and phytohormone production (Vessey, 2003; Barea et al., 2005). Such interactions may change nutrient dynamics, thus, altering plant's stress and indirectly affecting the accumulation of the rhizosphere phosphatase activities.

Likewise, supplying of Mo at 12 g kg^{-1} seeds may have facilitated the synthesis of ascorbic acid, thus, enabling physiological availability of Fe to plants for growth (Nicholas, 1975). Similarly, Mo could have catalyzed the N_2 fixation by *Rhizobium* in *P. vulgaris* L. by changing dinitrogen gas to ammonia, making it available for plant growth. There is also evidence that calcium (Ca) deficiency increase stress for plant growth (Speir and Ross, 1978; Bremner and Mulvaney, 1978). In this study, supply of Ca as lime at 3 t ha^{-1} decreased the secretion of acid and alkaline phosphatases suggesting that Ca availability was not limiting for plant growth. Higher mineral elements stress in the rhizosphere of legumes such as *P. vulgaris* L. have also been related to greater secretion of acid and alkaline phosphatase activities (Vance et al., 2003; Hammond et al., 2004; Makoi et al., 2010a). In fact Jones et al. (2003) reported increased secretion of acid phosphatase by 20-fold in lupins (*L. albus* L.) under P stress. Similarly, many plant species exude organic anions such as citric and malic acids in response to deficiency of several nutrients, including P, K, Fe, and Mn. Overall, these mechanisms leads to less stress for mineral elements in the rhizosphere soil, thus, lower secretion of acid and alkaline phosphatases.

The study also showed that *Rhizobium*, Mo and lime supply significantly reduced the levels of flavonoids and anthocyanins in shoots of *P. vulgaris* L. compared with control (Table 2). For example, *Rhizobium* inoculation lowered the secondary metabolites (Abs g DM^{-1}) from 0.210±0.005 (R-) to 0.174±0.009 (R+) and from 0.206±0.00 (R-) to 0.172±0.008 (R+) in glasshouse and field experiments respectively (Table 2). The reduced flavonoids and anthocyanins concentrations with *Rhizobium* inoculation suggest that stress for plant growth factors are not limiting. Lack of stress for plant growth factors in *P. vulgaris* L. could be attributed to greater accumulation of Ca, P, Mg, K, Zn and other mineral elements stimulated with different rhizosphere strains as similarly shown by Howell (1987). There is also evidence that most rhizobial strains produce siderophores, indole acetic acid (IAA), organic acids (OA), gibberellins, and cytokinins compounds which are used to mobilize Fe or solubilise P and other mineral elements (Law and Strijdom, 1989; Plessner et al., 1993; Antoun et al., 1998). However, when such mineral elements are found to be limiting, these secondary metabolites are raised in plant tissues indicating the stress for mineral elements encountered by the plant. That is why Bergman (1992) in his study involving different variety of plants reported accumulation of anthocyanins and attributed it to P and N deficiency symptoms. Similarly, the concentration of total phenolics was lower in P deficient plants (Juszczuk et al., 2004).

Supplying Mo at 12 g kg^{-1} and Ca^{2+} as lime at 3 t ha^{-1} significantly reduced the levels of flavonoids and anthocyanins compared with the other treatments (Table 2). The reduced levels of these secondary metabolites could be partly ascribed to the ability of Ca^{2+} supplied as lime to offset or decrease the stress of mineral elements probably by neutralizing the toxic effects of H^+, Al^{3+} and Mn^{2+} in the soil just as it increases soil pH so as to decrease the activities of Fe and Al oxides which are good sinks for Mo in soils (Mandal et al., 1998; Staley and Brauer, 2006). As a result, these mineral elements are made available for plant growth, thus, less stress, leading to low

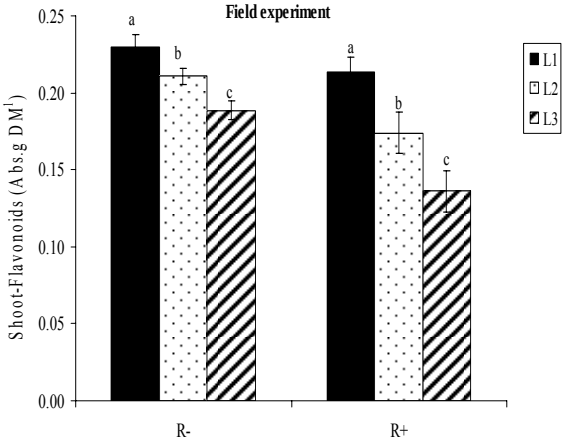

Fig. 2: Interactive effects of *Rhizobium* and lime on shoot flavonoids concentration in field experiment. (R-: Without *Rhizobium*, R+: With *Rhizobium*, L1: lime at 0 $t.ha^{-1}$, L2: lime at 2 $t.ha^{-1}$ and L3: lime at 3 $t.ha^{-1}$)

release of secondary metabolites such as flavonoids and anthocyanins. On the other hand, mineral element stress has been shown to have a notable effect on the concentrations of secondary metabolites in plant organs (Rengel, 1999). In this experiment, supply of Mo at 0 and 6 g kg^{-1} as well as lime at 0 and 2 t ha^{-1} showed relatively greater levels of flavonoids and anthocyanins compared with the other treatments (Table 2). The higher levels of these compounds could indicate stress for nutrients encountered by *P. vulgaris* L. during growth. Nitrogen (N) stress for example, may cause increased activity of L-phenylalanine ammonia-lyase (PAL), a key enzyme of phenylpropanoid metabolism leading to the biosynthesis and accumulation of secondary metabolites in *P. vulgaris* L. for example, as similarly reported previously in French bean (*P. vulgaris* L. cv Strike, Dakora and Phillips, 1996; Sánchez et al., 2000). There is also evidence that when P, Ca, Mg, S and Fe are deficient, secondary metabolites are stimulated and released in plant organs and in root exudates which then solubilise different mineral elements from unavailable sources and facilitate their uptake by plants for growth (Gerschenzon, 1983, Rengel, 2002). These compounds may also form stable chelate with Fe and Al present in insoluble Fe- and Al-phosphates, and in so doing, solubility of Fe and P is enhanced and therefore made available for plant growth (Dakora and Phillips, 2002), thus, lowering the stress for such mineral elements required for plant growth.

There was also significant interaction between *Rhizobium,* molybdenum and lime on flavonoids concentration in shoots of *P. vulgaris* L. (Figs. 1 and 2). Whether planted in field or in glasshouse, and regardless of Mo and lime supply, flavonoids concentration in shoots of *P. vulgaris* L. was consistently higher in without *Rhizobium* inoculation treatments compared with *Rhizobium* inoculation (Figs. 1 and 2). However, supply of Mo at 0 and 6 g kg^{-1} seeds as well as lime at 0 and 2 t ha^{-1} were significantly higher relative to the higher supply of Mo (12 g kg^{-1}) and lime (3 t ha^{-1}). These results suggest that regardless of inoculating or not inoculating *Rhizobium*, flavonoids concentration in shoots of *P. vulgaris* L. decreased as Mo and lime supply increased, and as a result, Mo and lime in this context controlled the flavonoids levels in shoots of *P. vulgaris* L (Figs. 1 and 2).

Conclusion

In conclusion, our result showed greatest acid and alkaline phosphatase activities as well as flavonoids and anthocyanins concentrations in control treatments involving no *Rhizobium* and zero Mo and lime. On the contrary, these parameters were lowest where supply of Mo and lime was highest. These results suggest that acid and alkaline phosphatase activities as well as flavonoids and anthocyanins concentrations are released when legumes such as *P. vulgaris* L. are confronted with low availability of mineral elements such as those tested in this study. Overall, this finding is important in the management of acid soils where several mineral elements are deficient including N, Mo and Ca.

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References

Antoun H, Beauchamp CJ, Goussard N, Chabot R and Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57-67

Bambara S and Ndakidemi PA (2010a) The potential roles of lime and molybdenum on the growth, N_2 fixation and assimilation of metabolites in nodulated legume: A special reference to *Phaseolus vulgaris* L. Afr J Biotechnol 8 (17): 2482-2489

Bambara S and Ndakidemi PA (2010b) Changes in selected soil chemical properties in the rhizosphere of *Phaseolus vulgaris* L. supplied with *Rhizobium* inoculants, molybdenum and lime. Sci Res Essays 5(7): 679-684

Bambara S and Ndakidemi PA (2010c) Effects of *Rhizobium* inoculation, lime and molybdenum on nitrogen fixation of nodulated *Phaseolus vulgaris* L. Afr J Microbiol Res 4(9): 682-696

Barea J-M, Pozo MJ, Azcón R, Azcón-Aguilar C (2005) Microbial co-operation in the rhizosphere**.** J Exp Bot 56:1761-1778

Bergmann W (1992) Nutritional Disorders of Plants: Development, Visual and Analytical Diagnosis. Gustav Fischer Verlag Jena, New York

Bozzo G, Raghothama K, Plaxton W (2002) Purification and characterization of two secreted purple acid phosphatase isozymes from phosphatase- starved tomato (*Lycopersicon esculentum*) cell cultures. Eur J Biochem 269:6278-6286

Bremner JM, Mulvaney RL (1978) Urease Activity in soils. In: Soil Enzymes (Burns RG Ed.), pp 149-196. Academic Press, New York

Dakora FD and Phillips DA (1996) Diverse functions of isoflavonoids in legumes transcend ant-microbial definitions of phytoalexins. Physiol Mol Plant Pathol 49:1-20

Dakora FD and Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35-47

Dick WA, Cheng L, Wang P (2000) Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol Biochem 32:1915-1919

Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In Biological Indicators of soil Health (Pankhurst CE, Doube BM, Gupta VVS. Eds), pp 121-156. CAB International: UK

Division of Chemical Services (1956) Analytical methods. Division of Chemical Services, Department of Agriculture, Pretoria

Dixon RA, Harrison MJ (1990) Activation, structure and organization of genes involved in microbial defence in plants. Adv Genet 28:165-234

Dixon RA, Steele C (1999) Flavonoids and isoflavonoids- gold mine for metabolic engineering. Trop Plant Sci 4:394-400

FAO (Food and Agricultural Organizations) (2001) World Soil Resources Reports, 289p

Gellatly KS, Moorhead GB, Duff SM, Lefebvre DD, Plaxton WC (1994) Purification and characterization of potato tuber acid phosphatase having significant phosphotyrosine phosphatase activity. J Plant Physiol 106:223-232

Gerschenzon J (1983) Changes in the level of plant secondary metabolites under water and nutrient stress. In: Phytochemical adaptation to stress (Timmermann BN, Steelink C, Loewus FA. Eds), Pp. 273-299. Plenum Press, New York

Guo Y, Terry ME, Roux SJ (1998) Characterization of a cytosolic phosphatase from pea plumules having significant protein tyrosine phosphatase activity. Plant Physiol Biochem 36: 269-278

Hammond JP, Broadley MR, White PJ (2004) Genetic responses to phosphorus deficiency**.** Ann Bot 94:323-332

Hodek P, Pavel T, Marie S (2002) Flavonoids potent and versatile biologically active compounds interacting with cytochromes. Chemical Biol Inter 139 (1):1-14

Howell RK (1987) *Rhizobium*-induced mineral uptake in peanut tissues. J Plant Nutr 10: 1297-1305

Jones DL, Dennis PG, Owen AG, van Hees PAW (2003) Organic acid behaviour in soils – misconceptions and knowledge gaps. Plant Soil 248: 31-41

Juszczuk IM, Anna W, Eligio M and Anna MR (2004) Changes in the concentration of phenolic compounds and exudation induced by phosphate deficiency in bean plants (*Phaseolus vulgaris* L.). Plant Soil 267:41-49

Law IJ, Strijdom BW (1989) Inoculation of cowpea and wheat with strains of Bradyrhizobium sp. that differ in their production of indole acetic acid. South Afr J Plant Soil 6: 161-166

Liebenberg AJ (2002) Dry bean Production. Printed and published by Department of Agriculture, Resource Centre, Directorate Agricultural Information Services, Private Bag X144, Pretoria, 0001 South Africa. p. 27. Available online on 15/09/2009: http://www.nda.agric.za/docs/drybeans2.pdf

Lindoo SJ, Caldwell MM (1978) Ultraviolet-B radiation induced inhibition of leaf expansion and promotion of anthocyanin production. Lack of the involvement of the low irradiation phytochrome system. Plant Physiol 61: 278-282

Lopez RS, Anali ER, Rao MA, Maria De La Luz Mora, Alvaer M, Gianfreda L (2007) Manganese and molybdenum affect acid phosphatases from potatoes. Acta Agr Scand B-S P 57:65-73

Lunze L, Kimani PM, Ngatoluwa R, Rabary B, Rachier GO, Ugen MM, Ruganza V, Awad Elkarim EE (2007) Bean Improvement for Low Soil Fertility Adaptation in Eastern and Central: In: Advances in integrated soil fertility research in Sub-Saharan Africa: challenges and opportunities (Eds Bationo E, Waswa B, Kihara J, Kimetu J), Dordrecht, The Netherlands: Springer pp. 325-332

Makoi JHR, Ndakidemi PA (2007) Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. Afr J Biotechnol 6:1358-1368

Makoi JHR, Ndakidemi PA (2008) Selected soil enzymes: examples of their potential roles in the ecosystem. Afr J Biotechnol 7:181-191

Makoi JHJR, Chimphango SBM and Dakora FD (2010a) Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (*Vigna unguiculata* L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L.). Crops Pasture Sci 61: 279-286

Makoi JHJR, Belane A, Chimphango SMB, Dakora FD (2010b) Flavonoids and anthocyanins in seed exudates enhance nodulation and defence in symbiotic cowpea (*Vigna unguiculata* L. Walp.). Field Crops Res. 118: 21-27

Mandal PS, Mandal LN (1998) Effect of molybdenum, phosphorus and lime application to acid soils on dry matter yield and molybdenum nutrition of lentil. J Plant Nutr 21:139-147

Mirecki RM and Teramura AH (1984) Effects of UV-B irradiance on soybean. V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol 74:475-480

Mloza-Banda HR, Ferguson AE, Mkandawire ABC (2003) The common bean (*Phaseolus vulgaris* L.) genetic resources in Malawi: Implications for conservation, utilization and production. Bunda J Agric Envron Sci Tech 1:1-13

Mukoko OZ, Galwey NW and Allen DJ (1995) Developing cultivars of the common bean (*Phaseolus vulgaris* L.) for southern Africa: bean common mosaic virus resistance, consumer preferences and agronomic requirements. Field Crop Res 40:165-177

Nadiya EL, Sandeno JM, McGrath D, Dick RP (2000) Integrative biological indicators for detecting changes in soil quality. Am J Alternative Agric 15: 26-36

Ndakidemi PA, Dakora FD (2003) Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. Funct Plant Biol 30:729-745

Ndakidemi PA (2005) Nutritional characterization of the rhizosphere of symbiotic cowpea and maize plants in different cropping systems. Doctoral degree Thesis. Cape Peninsula University of Technology, Cape Town, South Africa. p. 150

Ndakidemi PA (2006) Manipulating legume/cereal mixtures to optimize the above and below ground interactions in the traditional African cropping systems. Afr J Biotechnol 5:2526-2533

Nicholas DJD (1975) The function of trace elements in plants. Pages 181-198 in Nicholas DJD and Egan AR eds. Trace Elements in Soil-Plant-Animal Systems. Academic Press, New York.

Plessner O, Klapatch T, Guerinot ML (1993) Siderophore utilization by *Bradyrhizobium japonicum*. Appl and Environ Microbiol 59: 1688-1690

Rengel Z (1999) Physiological mechanisms underlying differential nutrient efficiency of crop genotypes. In Mineral nutrition of crops: mechanisms and implications. (Rengel Z Ed.), pp 227-265. The Haworth Press, New York, USA

Rengel Z (2002) Genetic control of root exudation. Plant Soil 245:59-70

Sánchez-Ballesta MT, Lafuente MT, Zacarias L and Granell A (2000) Involvement of phenylalanine ammonia-lyase in the response of Fortune mandarin fruits to cold temperature. Physiol Plantar 108: 382-389

Singh SP (1999) Common bean improvement in the twenty-first century. Kluwer Academic Publisher, 420 p

Soil Classification Working Group (1991) Soil classification: A taxonomic system for South Africa. Memoirs of the natural agricultural resources of South Africa. No. 15

Speir TW, Ross DJ (1978) Soil phosphatase and sulphatase. In: Soil Enzymes; (Burns RG Ed.). Pp 197-250. Academic Press: New York

Staley TE, Brauer DK (2006) Survival of a genetically modified rootcolonizing Pseudomonad and Rhizobium strain in an acidic soil. Soil Sci Soc Am J 70:1906- 1913

Steel RGD and Torrie JH (1980) Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw Hill, New York.

Sugiura Y, Tanaka H, Mino Y, Ishida T, Ota N, Inoue M, Nomoto K, Yoshioka H, Takemoto T (1981) Structure, properties and transport mechanism of Iron (III) complex of mugineic acid, a possible phytosiderophore. J Am Chem 103:6979- 6982

Tabatabai MA (1994) Soil enzymes. In: Weaver, RW, Augle S, Bottomly PJ, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds.), Methods of soil analysis. Part 2. Microbial and biochemical properties, No. 5. Soil Science Society of America, Madison. pp.775-833

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157: 423-447

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571-586

Wortmann CS, Lunze L, Ochwoh VA and Lynch JP (1995) Bean improvement for low fertility soils in Africa. Afr Crop Sci J 3(4):469-477

Wortmann CS, Kirkby RA, Aledu CA, Allen JD (1998) Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa, CIAT Cali, Colombia