African Journal of Agricultural Research Vol. 5(14), pp. 1804-1811, 18 July, 2010
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR09.584
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Phaseolus vulgaris response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa

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Accepted 28 April, 2010

The effects of *Rhizobium* inoculation, lime and molybdenum supply on yield and yield components of *Phaseolus vulgaris* L. were investigated in a split-split plot design in the greenhouse and field experiment. Results showed that *Rhizobium* application significantly improved the number of pods per plant, number of seeds per plant, 100-seed weight and seed yield. Furthermore, compared with the zero treatment control, molybdenum supply significantly increased the number of pods per plant, number of seeds per plant, 100-seed weight and seed yield. In general, these parameters were significantly increased with molybdenum supplied at the highest rate of 12 g per kg of seed. With regard to lime, significant increases were recorded in number of seeds per pod and seed yield. Application of lime at the highest rate (3 t lime per ha) was significantly superior to the control and 2 t of lime per ha. The combination of these supplies at different levels resulted in significant interactions in some parameters and thus indicating need for these inputs in the study area.

Key words: Beans, number of pods per plant, number of seeds per pod, 100-seed weight, interaction.

INTRODUCTION

Soil acidity is a major factor that affects plant growth in many countries (Xu et al., 2002; Bolan and Hedley, 2003; Ndakidemi and Semoka 2006; Godsey et al., 2007). Soil acidity is therefore, a main hindrance to the availability of bases such as Ca and Mg and other nutrients such as Mo and N may secondarily influence the growth and yield in legumes (Munns, 1970; Bell et al., 1989). These constraints may be ameliorated by supplying lime, molybdenum (Mo) and inoculation of legumes with the appropriate *Rhizobium*. Leguminous plants needs Ca, N, and Mo for their normal growth. Some of these elements play vital roles in different growth process. For instance, research evidence suggests that Ca, Mo and N deficiency in legumes can restrict plant growth through different mechanisms (Bonilla and Bolaños, 2009). For example, Ca^{+2} deficiency is known to restrict the amount of N_2 fixed in legumes, resulting in reduced plant growth due to inadequate nitrogen which is required as building blocks of proteins. On the other hand, plants with severe Ca deficiency have shown low levels of N in their tissues and this has always been associated with poor growth (Evans et al., 1950; Evans and Purvis, 1951; Marschner, 1995; Dutta, 2004).

Studies by Shoemaker et al. (1961), Adams and Evans (1962), Curtin et al. (1984), Edmeades et al. (1985) and Lucrecia et al. (1987) demonstrated that supply of Ca^{2+} through lime significantly increased plant growth and productivity. In an experiment done by Hartley et al. (2004), lime supply increased nodulation and yield of Serradella (*Ornithopus compressus*). The beneficial effects of liming on nodulation and plant growth most likely resulted from the enhanced conditions for seedling growth and nodulation. Interestingly, a study by Phillips et al. (1999) has also reported that rhizobia inoculants can stimulate growth and final yield of leguminous plants. It is well known that leguminous plants are very sensitive to Mo deficiency, but excess Mo also may impair growth, decreases plant biomass, seed yield and deteriorate the quality of seed (Liu and Yang, 2000; Kevresan et al.,

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2001; Nautiyal and Chatterjee, 2004). During the growth processes of plants, and legumes in particular, Mo is involved in a number of different enzymatic processes (Marshner, 1995; Vieira et al., 1998). For example, Mo is a constituent of nitrogenase enzyme, and it is also needed by *Rhizobium* bacterium during the fixation process (Vieira et al., 1998). Therefore, with this task, Mo has a positive effect on growth, yield, N content of foliage and roots, as well as nodule forming in leguminous plants (Kliewer and Kennedy, 1978; Togay et al., 2008). With regard to other aspects of plant nutrition, molybdo-enzymes are involved in N metabolism, improving qualities of ascorbic acid, soluble sugar, and chlorophyll concentrations (Zhao and Bai, 2001; Chen and Nian, 2004). Therefore, its deficiency may show overall reductions in plant growth, poor pod and/or grain development as well as exposing the plant to pest damage (Graham and Stangoulis, 2005). Although there is considerable literature on the beneficial effects of liming, Mo and *Rhizobium* inoculation on legume growth in other parts of the world (Staley and Brauer, 2006 Bottomley and Thies, 1995), site specific factors can yield different results. As Ca, Mo and N play important roles in legume growth, these mineral nutrients warrants further investigations to ascertain their effects on plant growth and development in common legumes grown by farmers in the Western Cape such as *P. vulgaris* L.

MATERIALS AND METHODS

Site location and description

The experiments were conducted in the glasshouse of the Cape Peninsula University of Technology, Cape Town Campus, from August 2008 to January 2009 and 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 rainfalls on the farm are 713.4 mm and mean annual temperatures range from 22.6°C during daylight to 11°C at night. The experimental site had a previous history of grape (*Vitis vinifera*) 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).

Experimental design

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

After ploughing and harrowing lime application was done 2 weeks before planting. Twelve hours before planting, seeds involving Mo treatments were soaked into molybdenum solution. The control was 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.

Plant harvesting and analysis

At physiological maturity, the plants in the two middle rows of each plot were counted and harvested for assessing grain yield. The border plants within each row were excluded. For yield components, 10 plants were sub-sampled from each plot to determine the number of pod per plant and number of seeds.pod^{-1}. All pods were manually threshed and allowed to dry to 13% moisture content. Grain yield was determined for each plot and 100-seed weight recorded.

Statistical analysis

The data from this experiment was analysed using the software of STATISTICA programme 2008 (StatSoft Inc., Tulsa, OK, USA). When significant differences were detected by the analysis of variance (ANOVA), Fisher's least significant difference was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

RESULTS

Effect of *Rhizobium* inoculation on yield components of *P. vulgaris* L

The results in Table 1 clearly demonstrate that *Rhizobium* inoculation had significant effects on yield and all the other yield components assessed in this study. All parameters measured (number of pods.plant^{-1}, number of seeds.plant^{-1}, 100-seed weight, and seed yield) were significantly increased with *Rhizobium* inoculation. For instance, the number of pods.plant^{-1} for the glasshouse and field experiments were increased significantly with *Rhizobium* inoculation by 13% for the glasshouse and 8% for field experiment relative to the uninoculated treatment (Table 1). In field experiment, the number of seeds.pod^{-1} in the inoculated treatment was 8% greater compared with uninoculated control. The 100-seed weight (g) was increased by 17.3% in the treatments supplied with *Rhizobium* compared with control. The grain yield (kg.ha^{-1}) of *P. vulgaris* L. were also significantly greater by 122% in plots inoculated with *Rhizobium* compared with the uninoculated control.

Effect of molybdenum on yield and yield components of *P. vulgaris* L

There was a significant response in yield and other yield components of *P. vulgaris L.* supplied with Mo at 0, 6 and 12 g.kg^{-1} of seeds (Table 1). In general, all parameters measured were significantly increased with Mo application relative to control (zero-level of Mo). In the

Table 1. Yield components of nodulated *P. vulgaris* L. supplied with *Rhizobium*, lime and molybdenum.

Treatments	Glasshouse	Field			
	No of pods plant $^{-1}$	No of pods plant $^{-1}$	No Seeds pod $^{-1}$	100-seed wt (g)	Seed yield (kg/ha)
Rhizobium					
-R	3.7±0.14b	3.8±0.13b	3.7±0.10b	15.0± 0.40b	758±52.5b
+R	4.2±0.12a	4.1±0.14a	4.0±0.01a	17.6± 0.40a	1679±51.9a
Molybdenum (g.kg^{-1})					
0	3.5±0.18c	3.5±0.16c	3.5±0.13b	14.7 ± 0.56 b	875±102.9c
6	4.1±0.14b	4.2±0.18b	4.0±0.00a	16.7 ± 0.41a	1237±99.2b
12	4.3±0.13a	30.2± 0.73a	4.0±0.00a	17.3 ± 0.53a	1544±99.7a
Lime (t.ha^{-1})					
0	3.9±0.20a	3.9±0.17a	3.7±0.12b	16.04 ± 0.6a	1082±115.9c
2	3.9±0.12a	4±0.1 a	3.8±0.07b	16.4 ± 0.4a	1205±104.8b
3	4.3±0.16a	4 ±0.2 a	3.9±0.04a	16.4± 0.58a	1369±117.9a
3- way ANOVA (F-Statistic)					
R	11.4**	3.9*	51.9***	34.1***	7748.3***
Mo	10.3***	6.9**	51.9***	12.7***	1364.7***
L	1.882NS	0.292NS	8.1***	0.287 NS	252.7***

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

glasshouse experiment, compared with the control, supplying 6 or 12 g Mo increased the number of pods.plant^{-1} by 17 and 23% respectively. In the field experiment, the number of pods.plant^{-1} were significantly greater in plots supplied with the highest rate of 12 g of Mo.kg^{-1} of seed and was followed by 6 g Mo.kg^{-1} of seeds (Table 1). Compared with the control treatment, applying 6 and 12 g Mo increase the number of pods.plant^{-1} by 20 and 763% respectively. The value of 100-seed weight (g) also increased significantly by 13.6 - 17.7% with the supply of Mo at 6 or 12 g Mo.kg^{-1} seeds respectively compared with the control.

Results from this study also showed that application of Mo at any level significantly increased grain yield (kg.ha^{-1}) of *P. vulgaris* L. For instance, supplying Mo at 6 and 12 g significantly increased the seed yield by 41 - 76.5% respectively compared with zero-molybdenum control (Table 1).

Effect of lime on yield and yield components of *P. vulgaris* L

In this experiment, the results in Table 1 demonstrate that lime had no significant effects on the number of pods.plant^{-1} and 100-seed weight. However, significant increases were recorded in number of seeds.pod^{-1} and seed yield. Application of lime at the highest rate (3 t lime.ha^{-1}) was significantly superior to the control and 2 t lime.ha^{-1}.

The values of grain yield increase significantly at each level of lime application. The highest yield was recorded by supplying lime at 3 t lime.ha^{-1}. As compared with the control treatment, the increase by applying 2 and 3 t lime.ha^{-1} was 11 and 27% respectively (Table 1).

Interactive effects of *Rhizobium*, molybdenum and lime

The results in Figure 1A-C showed significant interaction between *Rhizobium* and Mo only in the number of seeds.pod^{-1}, the 100-seed weight and the grain yield of *P. vulgaris* L. The lowest number of seeds.pod^{-1} was recorded in the control treatment (Figure 1A), whereas highest seed yields were found in treatments supplied with *Rhizobium* and different levels of Mo (Figure 1C). The *Rhizobium* x lime interaction was significantly different for the number of seeds per pod and the grain yield (Figure 2A-B). Greater yields were recorded in treatments involving *Rhizobium* inoculation and lime. Increasing lime levels progressively resulted in increased seed yield (Figure 2B). The results in Figure 3A-C shows that there was significant interaction between Mo and lime in number of pods.plant^{-1}, the number of seeds.pod^{-1}, the 100-seed weight, and the grain yield. Highest rates of Mo and lime resulted in greater grain yield values (Figure 3C). The interaction between *Rhizobium*, Mo and

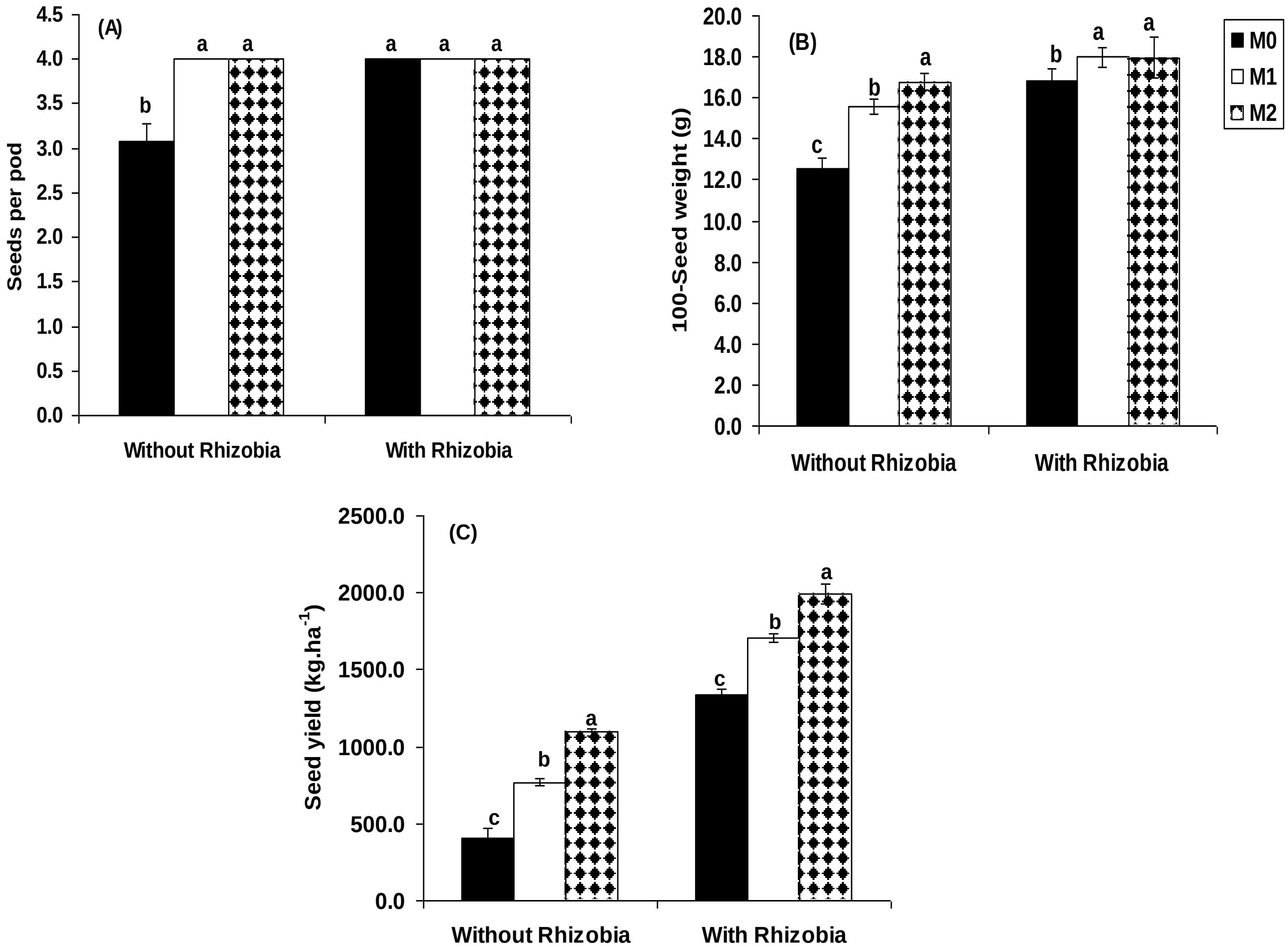

Figure 1. Interactive effects of Rhizobia and Molybdenum (Mo) on: (A) No of seeds per pod, (B) 100-seed weight, and (C) Seed yield. M0 = Control, M1 = 6 g Mo per kg seed, M2 = 12 g Mo per kg seed. Bars followed by similar letter are not significantly different.

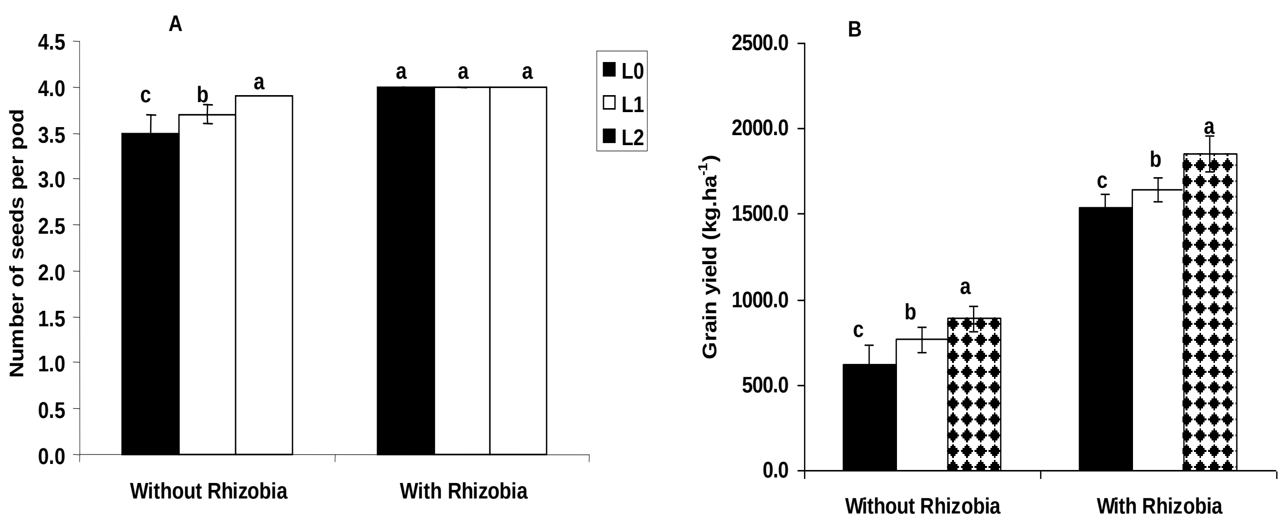

Figure 2. Interactive effects of Rhizobia and Lime on: (A) No of seeds per pod, (B) Seed yield. L0 = Control; L1 = 2t Lime per ha; L2 = 2t Lime per ha. Bars followed by similar letter are not significantly different.

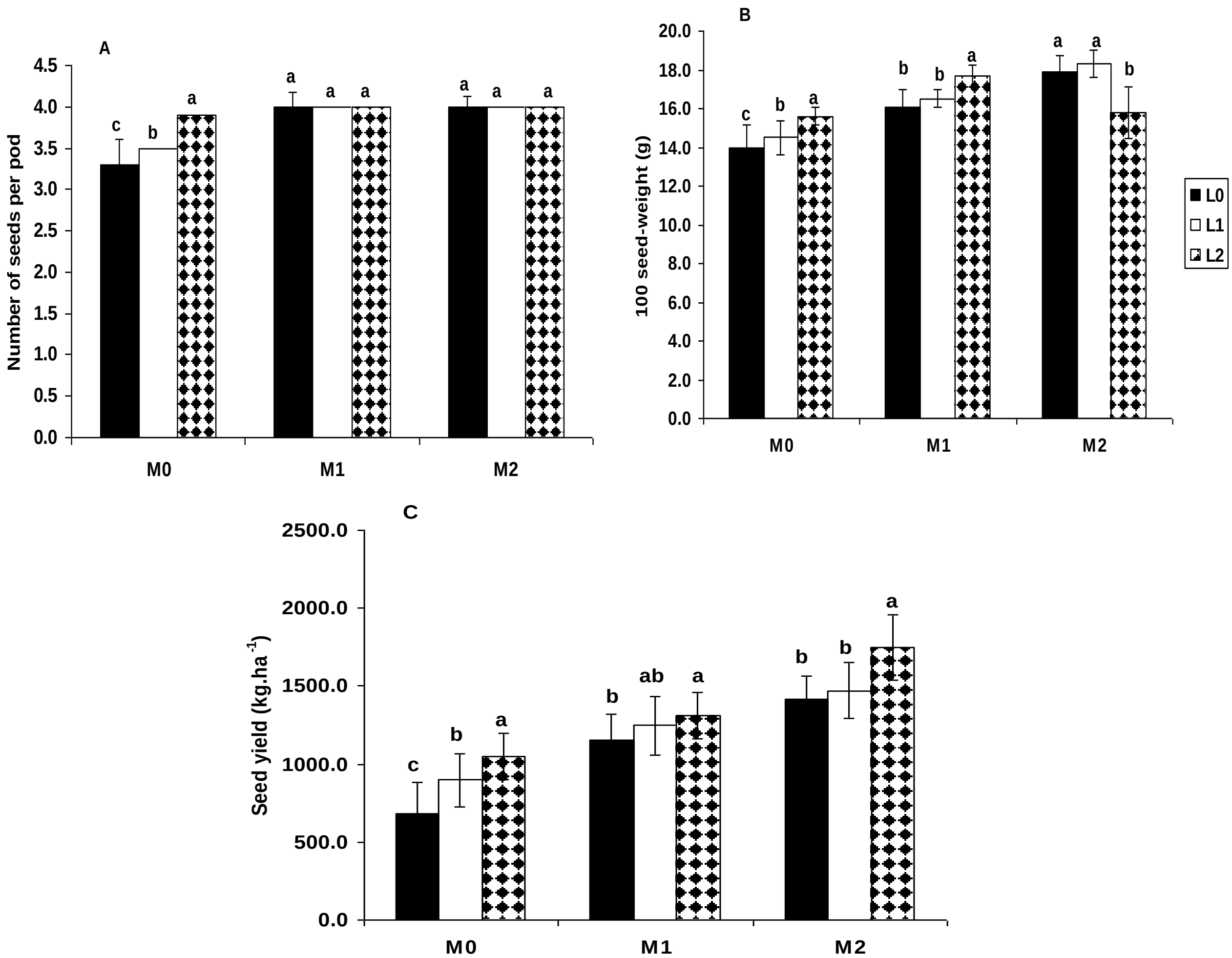

Figure 3. Interactive effects of Molybdenum (Mo) and Lime (L) on: (A) No of seeds per pod; (B) = 100-seed weight; and C = Seed yield. L0 = Zero lime; L1 = 2 t Lime per ha; L2 = 2t Lime per ha; M0 = Zero Mo; M1 = 6 g Mo per kg seed, M2 = 12 g Mo per kg seed. Bars followed by similar letter(s) are not significantly different.

lime was significant only in the grain yield (Figure 4). Higher yields were obtained in treatments including the combination of all these resources.

DISCUSSION

In this study, we combined lime, Mo and *Rhizobium* inoculation in order to maintain optimal soil pH and increase soil fertility and ultimately obtain a sustainable combination which will produce reasonable yield. This study reiterated the fact that *Rhizobium* inoculation was helpful in improving yield and yield components of *P. vulgaris* L. The treatments supplied with *Rhizobium* inoculation had positive responses in yield and other yield components (Table 1). The number of pods.plant^{-1} for both glasshouse and field experiments, the number of seeds.pod^{-1}, 100-seed weight (g) and grain yield (kg.ha^{-1}) of *P. vulgaris* L. increased significantly in the *Rhizobium* inoculated treatments as compared with the control. Such a significant effect of rhizobia inoculation on common bean has also been reported by other researchers (Munns, 1978; Keyser and Munns, 1979; Graham et al., 1982; Wood et al., 1984; Graham, 1992; Galloway et al., 1995; Peoples et al., 1995; Ndakidemi et al., 1998 and 2006). The higher yields obtained with inoculation indicates that the *Rhizobium* technology is efficient in supplying N to legumes as inorganic-N fertiliser and a better option for resource-poor farmer who can't afford to purchase expensive inputs. It is well established that leguminous plants in partnership with *Rhizobium* have the ability to convert the atmospheric nitrogen into usable forms (Ndakidemi et al., 2006). From this study, it is clear that *Rhizobium* inoculation is important and plays crucial role in improving plant growth and increasing the grain yield of *P. vulgaris* L. in the study site.

Molybdenum also played a significant role in improving some attributes of yield and yield components of *P.*

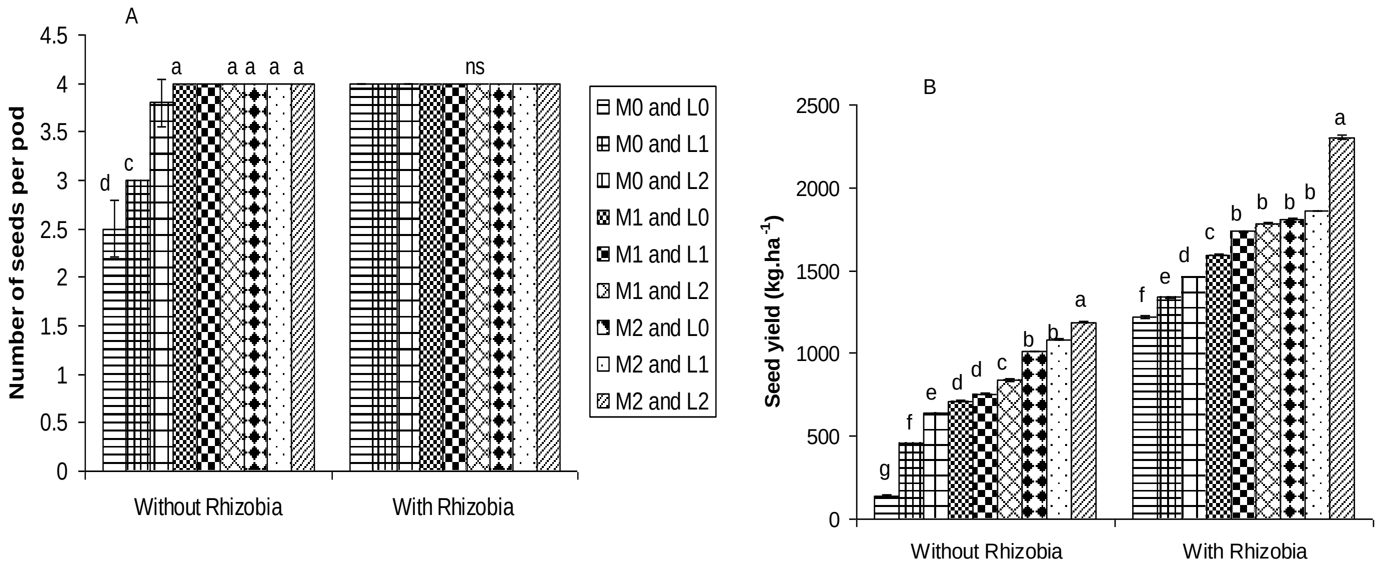

Figure 4. Interactive effects of Rhizobium, Molybdenum (Mo) and Lime (L) on: (A) No of seeds per pod; and (B) = Seed yield. L0 = Control; L1 = 2t Lime per ha; L2 = 3t Lime per ha; M0 = Zero Mo; M1 = 6 g Mo per kg seed; M2 = 12 g Mo per kg seed. Bars followed by similar letter are not significantly different.

vulgaris L. Results showed that plants supplied with 6 or 12 g of Mo.kg^{-1} seed significantly increased yield of *P. vulgaris* L. by 41 and 76.5% respectively compared with zero-control treatment (Table 1). Molybdenum is known to be a constituent of nitrogenase enzyme. Molybdo-enzymes are also involved in N metabolism and in improving qualities of ascorbic acid, soluble sugar, and chlorophyll concentrations in plants (Kliewer and Kennedy, 1978; Vieira et al., 1998; Zhao and Bai, 2001; Chen and Nian, 2004; Togay et al., 2008). Therefore, the supply of Mo in the study area which is known to be deficient in Mo (Thibaund, 2005) might have resulted into the observed positive effect on growth, yield and yield components (Table 1). Our results are consistent with other workers (Kliewer and Kennedy, 1978; de Yunda and Gonzalez, 1982; Vieira et al., 1998; Zhao and Bai, 2001; Chen and Nian, 2004; Togay et al., 2008) who reported positive results on growth and yield in other related legume species (Kliewer and Kennedy, 1978; Togay et al., 2008). The application of Mo in the study area was essential because it improved plant growth and increase the grain yield of *P. vulgaris* L. In our experiment, the application of lime at 2 or 3 t.ha^{-1}, improved the number of seeds.pod^{-1} and the grain yield of *P. vulgaris* L. Seed yield increased by between 11 and 27% compared with zero-lime control (Table 1). In similar studies involving other legumes by Shoemaker et al. (1961); Adams and Evans (1962); Curtin et al. (1984); Edmeades et al. (1985); Lucrecia et al. (1987) and Hartley et al. (2004), liming materials significantly increased plant productivity. The beneficial effects of liming on acidic soils such as those used in this study is most likely from the improved soil conditions through the neutralization of soil acidity and the improved Ca and Mg supply in the soil media.

A significant interactive effect was observed between *Rhizobium* inoculation and the Mo on the number of seeds.pod^{-1}, 100-seed weight (g), and grain yield. Good results were recorded in *Rhizobium* inoculated treatments in combination with the highest rate of Mo (Figure 1A-C), suggesting significant additive results by mixing these inputs. Given that Mo has a crucial role in N_2 fixation (Kliewer and Kennedy, 1978; Vieira et al., 1998; Togay et al., 2008), specifically as an important component of nitrogenase enzyme, it is possible that in combination with *Rhizobium* inoculation, Mo it played a critical function in N_2 fixation and this resulted in the observed results. The application of *Rhizobium* inoculation and lime interacted significantly in such a way that the number of seeds.pod^{-1} and the grain yield of *P. vulgaris* L. were increased. Better results were recorded in treatments involving *Rhizobium* inoculation and lime. Increasing lime levels progressively resulted in increased seed yield (Figure 2B). It may be suggested that the observed benefits were due to ability of these treatments to improve the nutrition on N (from rhizobia) and Ca and/or Mg (from lime). It is well established that acidic soil has low capability to support plant growth and are deficient in N, Ca and Mg (Ndakidemi and Semoka, 2006). Therefore, in this study there was a significant advantage of combining the two treatments together. Similar results were also reported in peanut by Simbajon and Duque (1987). Significant interactions also occurred with respect to Mo and lime in number of pods.plant^{-1}, number of seeds.pod^{-1}, 100-seed weight, and grain yield. The greater grain yield values were recorded into the highest rates of Mo (12 g of Mo.kg^{-1} seed) and lime (3 t.ha^{-1}) (Figure 3C). It is evident that the combination of Mo and lime was important in alleviating the Mo, Ca and or Mg stress in the study area as they have important role(s) to play in plant growth and development (Marschner, 1995). Research reports suggest that one major function of lime in acidic soils is to make Mo more available to plants (Quaggio et al., 2004). Similar results have also been reported in other leguminous species (Sahu et al., 1995; de Oliveira et al., 1998; Quaggio et al., 1998; Bailey and Laidlaw, 1999; Quaggio et al., 2004).

In the presence of all three treatments (*Rhizobium*, Mo and lime), a significant interaction was observed on the grain yield of *P. vulgaris* L. The greater grain yield was observed in the highest rates of Mo and lime in combination with *Rhizobium* inoculation (Figure 4). The improvement observed could be related to the amelioration effects of the limiting nutrients which were supplied as treatments in this study. Increasing levels of lime and Mo resulted in progressive increase in *P. vulgaris* L. seed yields. It is possible that supplying these inputs into the soil increased their content and finally improved the growth conditions. Coventry et al. (1985) also reported the ameliorating effects of *Rhizobium*, lime and Mo in clover in which the plant growth and yield were increased.

Conclusion

Rhizobium inoculation and the supply of Mo significantly improved yield and all yield components reported in this study. Better results were recorded in plots supplied with the highest rate of 12 g of Mo.kg^{-1} of seed and was followed by 6 g of Mo.kg^{-1} of seed. Lime application alone significantly improved number of seeds.pod^{-1} and the final seed yield. Significant interactive effects were reported by inoculating the soil with *Rhizobium*, and supplying Mo and lime, indicating the need for these inputs in the study area.

ACKNOWLEDGEMENT

This study was supported by Belgium Technical Cooperation (BTC/CTB) and Cape Peninsula University of Technology through University Research Fund (RP 03).

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