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Effect of Rhizobia Inoculation, Farm Yard Manure and Nitrogen Fertilizer on Nodulation and Yield of Food Grain Legumes

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Abstract: Field experiments were conducted to investigate the response of grain legumes to rhizobia inoculation, farmyard manure and inorganic fertilizer nitrogen. The grain legumes were common bean (*Phaseolus vulgaris* L. var GLP 2), lima bean (*Phaseolus lunatus* L.), green gram (*Vigna radiate* L.) and lablab (*Lablab purpureus* L.). The experimental design was a randomized complete block design with split plot arrangement and replicated thrice. Parameters determined were the number of nodules and nodule dry weight per plant, seed yield and yield components. Nitrogen fertilizer application significantly reduced the number of nodules in most of the legume species. In contrast, rhizobia inoculation increased number of nodules and nodule dry matter in most species but this was not translated into increase in plant growth or grain yield. Application of manure improved nodulation and grain yield only in the short rains. However, fertilizer application significantly increased dry matter in both seasons and total grain yield during short rains. The study indicated that the effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on grain legumes is variable depending on species, parameter being measured and other environmental factors.

Key words: Dry matter, grain legumes, grain yield, nitrogen source, nodulation

INTRODUCTION

Declining soil fertility and high fertilizer cost are major limitations to crop production in smallholder farms in Kenya (Maobe *et al*., 2000; Ojiem *et al*., 2000; Cheruiyot *et al*., 2001; Chemining'wa *et al*., 2004). This has been augmented by intensification of agriculture coupled with the reduction in farm sizes (Saha and Muli, 2000). Requirements for nitrogen exceed any other major nutrients and rarely do soil have enough of this nutrient to produce high sustainable yields (Mkandawire *et al*., 1998; Woldeyohannes *et al*., 2007). The quantity of nitrogen needed for agriculture is projected to increase and this would lead to greater environmental degradation (Tilman, 1999). Reduced dependence on nitrogen fertilizer and adopting farming practices that favour the more economically viable and environmentally prudent nitrogen fixation will benefit both agriculture and the environment (Woldeyohannes *et al*., 2007; Zaman-Allah *et al*., 2007). There are several options that are available to manage nitrogen in farmer's fields and chemical fertilizers are often considered to be an immediate answer to current nutrient deficiencies in soil (Woomer *et al*., 1997; Chemining'wa *et al*., 2004; Gentili *et al*., 2006). Unfortunately, commercial nitrogen fertilizers are expensive and out of reach of most small-scale farmers. As a result, cheaper sources of nitrogen need to be sought if yields are to be sustained to attain food security.

The use of organic inputs as external nutrient sources has been advocated as a logical alternative to expensive fertilizers in Africa (Reinjitjes *et al*., 1992; Ganeshamurthy and Sammi Reddy, 2000). In addition, in countries where nitrogen fertilizers are imported and the technology for manufacturing them is limited or too expensive to afford, a greater demand is being made on alternative and inexpensive sources of nitrogen Mwangi (1994). Biological materials may offer a solution in alleviating soil fertility problems and hence increase in crop production. The use of farm-derived sources such as crop residues, compost, manures, household wastes, has commonly been used in the management of soil fertility (Kimani *et al*., 1998). Animal manure and compost are beneficial in soil because they can increase the water holding capacity and cation exchange capacity (Nandwa, 1995). Biological Nitrogen Fixation (BNF) has been used in farming systems to cut down on fertilizer expenses (Mwangi, 1994; Shamseldin and Werner, 2004; Shamseldin, 2007; Vinuesa *et al*., 2003). Inoculation with an effective and persistent rhizobium strain has numerous

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advantages, which include non-repeated application of nitrogen fertilizers and higher pod yield due to increased nodulation (Sanginga *et al*., 1994). It has been reported that rates of N_2 fixation of 1 to 2 kg N ha^{-1} growing season day^{-1} is possible in most legumes in tropical cropping systems (Giller, 2001).

Therefore, improved nitrogen management is needed to optimize economic returns to farmers and minimize environmental concerns associated with nitrogen use (Bundy and Andraski, 2005). This study was therefore conducted to assess the response of food grain legumes to fertilizer nitrogen, farmyard manure and rhizobia inoculation with respect to growth, nodulation and yield.

MATERIALS AND METHODS

Experimental design and treatments: Field experiments were carried out during the short rains (November 2005 to February 2006) and long rains (April to June 2006) at the Faculty of Agriculture, University of Nairobi. The experiments were laid out in a randomized complete block design with a split plot arrangement and replicated three times. The legumes formed the main plots and the nitrogen sources were the subplots, each measuring 3×2 m with a 1 m alley between the plots and blocks to minimize inter-plot interference. The grain legumes were common bean (*Phaseolus* vulgaris L. cv GLP 2), lima bean (*Phaseolus lunatus* L.), green gram (*Vigna radiata* L.) and lablab (*Lablab purpureus*). The legumes were supplied with either 46 kg ha^{-1} of nitrogen, or 8 T ha^{-1} of farmyard manure or rhizobia inoculant. Control plots did not receive any of the nitrogen source treatments. Phosphorous (20 kg $plot^{-1}$) in the form of Triple Super Phosphate (TSP; 45% P_2O_5) was applied uniformly in all the plots. Rhizobia inoculation was at the rate of 100 g of inoculant for 15 kg of seeds wetted with 300 mL of water. The moist seeds were thoroughly mixed with the inoculant in the shade and sown immediately. Two to three seeds were placed in the furrows at the recommended spacing of 30×15, 45×35, 30×10 and 75×45 cm for common bean, lima bean, green gram and lablab, respectively. The plants were thinned to one plant per hill after emergence. The crops were sprayed with Dimethoate 40% EC and copper oxychloride WP for the control of insect pests and diseases.

Determination of nodule number, dry weight accumulation and seed yield: Three plants were randomly selected from each plot and dug out at 7 weeks after emergence. The plants were separated into shoots and roots. Soil was carefully washed from the roots. The nodules were picked from the roots and their numbers recorded for each plant. The shoots, roots and nodules were oven-dried at 70°C for 48 h for dry weight determination. At pod maturity, ten plants were randomly selected from each plot and tagged for yield assessment. Yield parameters determined were number of pods per plant and total grain yield. Seed yield per hectare was extrapolated from the seed yield per plot.

Data analysis: All the data were subjected to Analysis of Variance (ANOVA) using the PROC ANOVA Procedure of Genstat (Lawes Agricultural Trust Rothamsted Experimental Station, 1998, version 8) and differences among the treatment means compared using Fisher's Protected LSD test at 5% probability level.

RESULTS AND DISCUSSION

Legume species, N source and their interactions had a significant ($p\leq0.05$) effect on the number of nodules per plant in both seasons. During the long rains, fertilizer application significantly reduced the number of nodules per plant in lablab and common bean but had no significant effect on green gram and lima bean (Table 1). Manure application had no effect on number of nodules per plant for all the legume species during the long rains. In contrast, rhizobia inoculation significantly increased number of nodules per plant in lablab and common bean but had no significant effect on green gram and lima bean. During the short rains, fertilizer application had no effect on number of nodules per plant but manure application and rhizobia inoculation significantly increased the number of nodules per plant in all the legumes species except lima bean.

During the long rains, significant ($p\leq0.05$) differences in nodule dry weight per plant were observed among the legumes species and the N sources. Among

Table 1: Mean number of nodules per plant of grain legume species under different nitrogen sources during the long and short rain seasons of 2005

Legume	Fertilizer	Manure	Rhizobia	Control	Legume means
Long rains					
Lablab	4.3	8.1	11.6	8.0	8.0
Common bean	5.4	10.1	11.1	8.2	8.8
Green gram	1.4	4.8	5.1	3.8	3.8
Lima bean	1.6	2.8	2.5	2.9	2.4
N-source means	3.2	6.5	7.6	5.7	
Short rains					
Lablab	7.5	9.4	11.8	7.7	9.1
Common bean	8.3	10.0	12.5	8.8	9.9
Green gram	3.8	5.5	5.6	4.3	4.8
Lima bean	2.3	2.1	2.7	1.8	2.2
N-source means	5.5	6.8	8.2	5.6	
		LSD $_{(p\leq0.05)}$ Long rains		LSD $_{(p\leq0.05)}$ Short rains	
Legume		1.0		0.8	
N-source		1.4		0.4	
Legume×N-source		2.6		1.0	
CV (%)		29.7		8.0	

Table 2: Mean nodule dry weight per plant (g) of grain legume species under different nitrogen sources during the long and short rain seasons of 2005

Legume	Fertilizer	Manure	Rhizobia	Control	Legume mean
Long rains					
Lablab	37.8	62.4	105.6	61.1	66.7
Common bean	19.9	53.3	68.6	25.6	41.8
Green gram	7.80	38.0	45.8	12.2	25.9
Lima bean	14.3	11.1	17.8	13.3	14.1
N-source means	19.9	41.2	59.4	28.0	
Short rains					
Lablab	154.4	189.7	246.6	171.1	190.5
Common bean	67.0	96.1	114.7	68.8	85.8
Green gram	44.5	48.1	52.0	37.8	45.6
Lima bean	19.0	19.4	20.1	18.3	19.2
N-source means	70.4	88.3	108.3	74.0	
		LSD $_{(p\leq0.05)}$ long rains		LSD $_{(p\leq0.05)}$ short rains	
Legume		20.1		33.9	
N-source		18.2		11.4	
Legume×N-source		NS		36.6	
CV (%)		27.1		15.8	

NS: Not significant at 5% probability level

Table 3: Mean shoot dry matter (g m^{-2}) of grain legume species under different nitrogen sources during the long and short rain seasons of 2005

Legume	Fertilizer	Manure	Rhizobia	Control	Legume means
Long rains					
Lablab	19.6	17.5	18.1	18.0	18.3
Common bean	156.5	135.0	141.4	138.2	142.8
Green gram	74.8	62.8	62.2	58.7	64.6
Lima bean	36.8	30.3	33.0	30.3	32.6
N-source means	71.9	61.4	63.7	61.3	
Short rains					
Lablab	11.1	12.4	13.6	12.4	12.4
Common bean	98.0	91.9	97.4	93.3	95.2
Green gram	52.4	50.0	48.9	43.3	48.7
Lima bean	21.1	24.1	24.8	21.0	22.7
N-source means	45.7	44.6	46.2	42.5	
		LSD $_{(p\leq0.05)}$ Long rains		LSD $_{(p\leq0.05)}$ Short rains	
Legume		5.3		11.8	
N-source		7.5		NS	
Legume×N-source		NS		NS	
CV (%)		13.7		13.2	

NS: Not significant at 5% probability level

Table 4: Mean root dry weight (mg) per plant of grain legume species under different nitrogen sources during the long and short rain seasons of 2005

Legume	Fertilizer	Manure	Rhizobia	Control	Legume means
Long rains					
Lablab	313.0	282.0	335.7	252.3	295.8
Common bean	316.7	314.7	350.7	269.0	312.8
Green gram	177.7	141.0	166.3	159.3	161.1
Lima bean	321.7	222.0	256.3	287.7	271.9
N-source means	282.2	239.9	289.8	242.1	
Short rains					
Lablab	273.3	258.3	238.3	281.7	285.4
Common bean	273.3	263.3	276.7	269.0	261.7
Green gram	122.0	143.3	131.3	103.3	125.0
Lima bean	298.3	268.3	276.7	248.3	272.9
N-source means	241.8	233.3		216.7	
		LSD $_{(p\leq0.05)}$ Long rains		LSD $_{(p\leq0.05)}$ Short rains	
Legume		52.7		38.5	
N-source		NS		NS	
Legume×N-source	NS	NS			
CV (%)		10.1		8.2	

NS: Not significant at 5% probability level

Table 5: Mean number of pods per plant of grain legume species under different nitrogen sources during the long and short rain seasons of 2005

Legume	Fertilizer	Manure	Rhizobia	Control	Legume means
Long rains					
Lablab	170.7	159.0	161.0	156.0	159.2
Common bean	9.9	8.7	9.3	9.1	9.2
Green gram	6.8	7.1	9.9	6.6	7.6
Lima bean	41.8	31.3	35.6	37.8	36.6
N-source means	57.3	54.3	54.0	52.4	
Short rains					
Lablab	99.3	94.0	97.0	91.1	95.4
Common bean	8.9	8.0	8.2	7.0	8.0
Green gram	8.8	9.7	8.8	5.6	8.2
Lima bean	18.8	16.4	17.4	13.2	16.5
N-source means	33.9	32.0	32.9	29.2	
		LSD $_{(p\leq0.05)}$ Long rains		LSD $_{(p\leq0.05)}$ Short rains	
Legume		21.3		7.1	
N-source		5.0		3.8	
Legume×N-source		NS		NS	
CV (%)		20.1		11.1	

NS: Not significant at 5% probability level

the N sources, rhizobia inoculation and manure application resulted in the highest nodule dry weight (Table 2). The highest nodule dry weight per plant was recorded in lablab where as lima bean had the lowest nodule dry weight per plant. During the short rains, legume species, N source and their interactions had a significant ($p\leq0.05$) effect on nodule dry weight per plant. Fertilizer and manure application had no significant effect on number of nodules per plant in all the legumes tested whereas rhizobia inoculation increased nodule dry weight of lablab and common bean but not that of green gram and lima bean.

The interaction between the legume species and the N source on shoot dry matter was not significant ($p\leq0.05$) in both seasons (Table 3, 4). The N source had no significant ($p\leq0.05$) effect on number of pods per plant and number of seeds per pod in both seasons. During the short rains, fertilizer treated plants had significantly higher number of pods per plant (Table 5). However, significant differences between the N-sources on grain yield of the legume species was noted in the short rains but not in the long rains. Overall, manure and fertilizer application resulted in significantly higher grain yield with fertilizer having a significantly superior effect to manure application (Table 6). However, rhizobia inoculation had no significant effect on grain yield.

The study found that N fertilizer application significantly reduced number of nodules and nodule dry weight per plant. Inhibitory effects of added nitrogen fertilizer to nodulation and nitrogen fixation have been reported by Chemining'wa *et al.* (2004), Gentili and Huss-Danell (2003), Gentili *et al.* (2006), Laws and Graves (2005),

Table 6: Mean total grain yield (kg ha^{-2}) of grain legume species under different nitrogen sources during the long and short rain seasons of 2005

Legume	Fertilizer	Manure	Rhizobia	Control	Legume means
Long rains					
Lablab	3.040.	2.784	306	2.989	2.944
Common bean	2.723	2.628	2.650	2.430	2.608
Green gram	157	258	264	180	215
Lima bean	3.047	2.904	2.645	2.856	2.863
N-source means	2.242	2.144	2.155	2.089	
Short rains					
Lablab	1.255	1.163	1.056	1.057	1.133
Common bean	1.804	1.836	1.796	1.501	1.734
Green gram	356	350	365	274	365
Lima bean	2.600	2.829	2.368	2.333	2.532
N-source means	1.775	1.544	1.211	1.292	
		LSD $_{(p\leq 0.05)}$ Long rains		LSD $_{(p\leq 0.05)}$ Short rains	
Legume		377.1		472.2	
N-source		NS		207.3	
Legume×N-source		NS		NS	
CV (%)		8.7		16.5	

NS: Not significant at 5% probability level

Oliveira *et al*. (2004), Pons *et al*. (2007) and Taylor *et al*. (2005). Chui *et al*. (1985) observed that the application of N fertilizer caused nodule degeneration on French beans. In addition, Gentili *et al*. (2006) reported that high N levels inhibited early cell divisions in the cortex of *Alnus incana* there by inhibiting nodulation. However, a recent study by Hristozkova *et al*. (2007) established that foliar application of nutrients on pea reduced the inhibitory effect on the root nodulation and nitrogen assimilator enzyme activities due to the molybdenum shortage when the plants were inoculated with *Rhizobium leguminosarum Bv. Vicue*.

Manure application slightly increased the number of nodules relative to the control. This was probably due to the slow mineralization of manure hence slow nitrogen release. In addition, the additional phosphorous present in the manure perhaps resulted in the positive effect of manure on nodulation. Phosphorous and farmyard manure have been reported to improve both the total and active nodules and nodule dry weight (Floor, 1985; Ganeshamurthy and Sammi-Reddy, 2000).

Rhizobia inoculation increased number of nodules and nodule dry weight per plant for most species but the increase in nodulation was neither translated to shoot and root dry matter accumulation nor to the grain yield. The legumes had nodules that were equally effective even without inoculation. This indicates that the soils contained indegenous rhizobia that nodulated the grain legume species (Chemining'wa *et al*., 2004). In addition, negligible nodulation was reported in lima bean and green gram even with inoculation. This suggests that rhizobia strains that form effective nodules with these legume species are absent in the soil and the inoculant strain used may not have been effective. Earlier study has suggested that inoculation does not always enhance nodulation (Chemining'wa *et al*., 2004). However, Huang and Erickson (2007) and Zaman-Allah *et al*. (2007) reported increased root nodule mass and root biomass on pea, lentil and bean after inoculation with different strains of *Rhizobium*. However, a strong variation was observed among the rhizobial strains based on their origin. Rhizobial strains of different origins vary in their symbiotic efficiency (Zaman-Allah *et al*., 2007).

In the present study, fertilizer application improved shoot dry matter in the long rains. The non-significant effect on the number of pods per plant and grain yield may have been due to the fact that adequate amount of nitrogen was probably present in the experimental site. Field trials conducted in Western Kenya by Amos *et al*. (2001) found that nitrogen fertilizer only increased the seed number per plant but not stand count after emergence, pod number per plant and grain yields of the common bean. Dry matter production and seed yield of soybean were increased significantly by the application of farm yard manure (Ganeshamurth and Sammi Reddy, 2000) while, Huang and Erickson (2007) reported increased shoot biomass on pea and lentil after inoculation with three strains of *Rhizobium leguminosarum* bv. *Viceae*.

All the nitrogen sources had no effect on total grain yield except during the short rains when fertilizer and manure treatments resulted in higher yields compared to rhizobia inoculation and the control. Seed yield has been observed to be one of the more stable morphological characteristics of many plant species (Chmielewski and Ruit, 2002). Manure contains high amount of organic matter which increases the moisture retention of soil and improves dissolution of nutrients particularly phosphorus (Nyende, 2001; Olupot *et al*., 2004). It also improves soil structure and in turn soil porosity. This allows better root growth and hence better nutrient uptake. In addition, applications of readily decomposed organic manure have been shown to improve crop tolerance to root rots (CIAT, 1992; Mutitu *et al*., 1989) and hence crop yield. The positive response of legumes to manure has also been attributed to the quantity of manure N already available for the plants, amount of N that becomes available after mineralization during the season, release and availability of phosphorus, potassium and microelements (Bocchi and Tano, 1994). Addition of fertilizer or organic manure may affect the root rot pathogens, either directly or indirectly (Huang and Erickson, 2007; Otsyala *et al*., 1998).

Lack of significant yield improvement by rhizobia inoculation has been documented by Chemining'wa *et al*. (2004), Dunigra *et al*. (1984) and Howle *et al*. (1987). The lack of response due to inoculation is attributed mainly to the presence of native effective strains of rhizobia in the soil (Ham *et al*., 1971), soil pH (Vinuesa *et al*., 2003; Shamseldin and Werner, 2004, 2005; Shamseldin, 2007), high soil nitrogen, cultivar and strain interaction (Cadwell, 1996; Payakapong *et al*., 2004) and drought (Swaine *et al*., 2007; Woldeyohannes *et al*., 2007). The results of the study indicated that rhizobia inoculation and manure application would be beneficial in improving productivity of grain legumes both in terms of nodulation and yield. This is important for small scale farmers who can not afford inorganic fertilizers.

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