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Effect of *Rhizobium* inoculation and nitrogen fertilizer application on growth, nodulation and yield of two garden pea genotypes

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Key words: Rhizobia, ambassador, nodules, shoot dry matter, plum, photosynthetically active radiation

1 SUMMARY

Nitrogen is the most limiting nutrient in smallholder garden pea farms in Kenya and can be corrected by application of inorganic fertilizers and *Rhizobium* inoculation. A study was conducted to compare the effects of *Rhizobium* inoculation and nitrogen fertilizer application on nodulation and yield of two garden pea varieties grown for local (variety Plum) and export (variety Ambassador) markets. Field experiments were conducted at University of Nairobi's Field Station in 2007 short and long rains. Varieties Plum and Ambassador were either inoculated with a commercial strain of *Rhizobium leguminosarum* bv. *viciae*, supplied with 30 or 60 kg N ha^{-1} or without any treatment. Inoculation and N-fertilizer enhanced shoot dry matter, but had no effect on grain yield. Plots receiving 60 kg N ha^{-1} intercepted more photosynthetically active radiation than non-treated control plots. *Rhizobium* inoculation increased number of active nodules and nodule dry matter. Plum variety accumulated more nodule and shoot biomass than Ambassador. Variety Ambassador had longer and more seeds per pod than Plum while the converse was the case in number of pods plant^{-1}. **Nodulation observed in control plots indicated that native pea rhizobia in Kabete soils are compatible with Plum and Ambassador garden pea varieties. Rhizobia inoculation of garden pea can yield similar shoot biomass as nitrogen application. Nitrogen fertilizer increased shoot dry matter, leaf area index and PAR interception by garden pea. Increases in above ground biomass and nodulation due to rhizobia inoculation and nitrogen fertilizer application were not translated into increased pod and grain yield. Growth and nodulation responses to inoculation and nitrogen fertilization were dependent on the garden pea genotype, hence the need to investigate the differential response of Plum and Ambassador It is suggested that a study be conducted to determine the effect of *Rhizobium* inoculation and nitrogen fertilization on a broad range of locally grown garden pea genotypes.**

2 INTRODUCTION

Garden pea production has gained popularity in Kenya over the recent past, and has attracted smallholder farmers who produce both for local and export market. Market reports show that the volume of garden pea exports has been steadily rising from zero in the early 1990s to about 2,065 tonnes in the year 2006 (HCDA, 2007). The European Union is the largest importer of peas (garden pea, snap and snow pea) with United Kingdom having the largest market share of about 36% (HCDA, 2011).

One of the major constraints to garden pea production in Kenya is low soil fertility. Nitrogen is the most limiting nutrient in smallholder farms (Chemining'wa et al., 2007) and can be corrected by application of inorganic fertilizers. Small-scale farmers are however constrained by limited financial resources to purchase such inputs (Odame, 1997). Farmyard manure can be a good nitrogen source, but poor yield responses after its application have been reported (Wanjekeche *et al*., 2002). This has been attributed to its poor quality, especially its high carbon to nitrogen ratio if improperly composted. Palm *et al.* (2000) analysed the nutritional status of organic manures and found that most of them had less than 1% of total N. In addition, they are not available in adequate quantities and have limited commercial value in Kenya. Biological nitrogen fixation, which is enhanced by seed inoculation with commercial strains of rhizobia, offers a cost-effective option. The cost of inoculating 10 kg of seeds required per hectare is about US$4 (KES 320) compared to nitrogen fertilizer US$45 (KES 3,600) required over the same area. In a review of published work on rhizobia inoculation, Elhassan *et al*. (2010) observed that financial gains due to inoculation far exceeded the use of N fertilizer. In addition, the use of *Rhizobium* inoculants enhances environmental safety (Neeraj *et al.,* 2009). Rabbani *et al*. (2005) reported that *Rhizobium* inoculation and P application improved garden pea productivity in low soil fertility conditions. Response of pea to inoculation is obtained in soils with low soil nitrogen, pH close to neutral, low population of native rhizobia, and in soils with no previous legume cultivation history (Vargas *et al*., 2000; Chemining'wa *et al.,* 2007; Erman *et al*., 2009). Response of garden pea to rhizobia inoculation is genotype dependent (Ali *et al.,* 2008). The objective of this study was to compare the effects of *Rhizobium* inoculation and nitrogen fertilizer on growth, nodulation and yield of two garden pea (*Pisum sativum* L.) genotypes grown for local (Plum variety) and export (Ambassador variety) markets.

3 MATERIALS AND METHODS

3.1 Experimental site: A field experiment was carried out at the University of Nairobi's Field Station in 2007 long and short rain seasons. The site is situated on latitude 1° 15" S, longitude 36° 44" E and altitude of 2089 m above the sea level. The site has bimodal distribution of rainfall with long rains from early March to late May and short rains from October to December. Mean annual maximum and minimum temperatures are 23°C and 13°C, respectively. Average annual rainfall is 993 mm, and the agro-ecological zone is LH2-3 (Jaetzold *et al.,* 2006). Prior to planting, soils were analysed at the National Agricultural Research Laboratories (Kenya), for soil pH, organic C, N and P which were 5.22, 2.4%, 0.28% and 0.29 ppm, respectively.

3.2 Experimental design, treatments and crop husbandry: The treatments consisted of pea (varieties Ambassador and Plum) inoculated with commercial rhizobia, uninoculated pea supplied with nitrogen fertilizer (30 kg N ha^{-1} or 60 kg N ha^{-1}) and control with neither rhizobia nor nitrogen fertilizer application. The varieties used in the study were selected based on growth characteristics and market class. Ambassador is an early maturing dwarf variety grown for export market and sometimes for the local market, while Plum is a late maturing tall variety grown for the local market. The commercial pea *Rhizobium* strain used was obtained from the Soil Microbiology Laboratory of the University of Nairobi, and applied as a seed dress. As a precaution against spread of *Rhizobium* to non-inoculated plots at planting, inoculated plots received treatments after the rest. Nitrogen was applied in form of calcium ammonium nitrate (CAN) in two splits; one at planting (starter nitrogen) and the other at four weeks after crop emergence. Triple super phosphate fertilizer was applied to all the plots at the rate of 46 kg P_2O_5 ha^{-1}. The treatments were laid out in a randomized complete block design with a factorial arrangement, and replicated three times. Plots of size 3 m by 2.5

m with 1m wide paths were used. Peas were sown 50 cm apart in rows, at intra row spacing of 10 cm. The fields were kept weed free by hand weeding. Beta-cyfluthin (Bulldock®) was applied at the rate of 50 ml per 100 litres of water, to prevent major insects like bollworms and thrips, while fungicide bupirimate (Nimrod®) was applied at the rate of 60 ml/ 100 litres of water, to prevent spread of diseases like powdery mildew. Training of pea stems was done two weeks after emergence to avoid crop contact with soil that could contain disease-causing pathogens.

3.3 Data collection: Data collected included nodule number, nodule dry matter, leaf area index, shoot dry matter, photosynthetic active radiation (PAR) interception, fresh pod weight, pod length, number of seeds per pod, 100 seed weight, total fresh seed weight and number of pods per plant. Five pea plants were randomly selected and carefully uprooted from each plot at 7 and 10 weeks after crop emergence. Soil was then washed off the roots in running water and nodules removed and counted. Each nodule was dissected and presence of pink coloration used to distinguish between those actively fixing nitrogen from the inactive ones. Roots were separated from the shoots. Nodules and shoots were oven- dried at a temperature of 60°C and dry weights were determined. Leaf area was determined using the cork borer method. Leaf area index was then calculated as leaf area per plant/ground area per plant (Malash *et al*., 2005). Photosynthetically active radiation (PAR) interception was measured in each plot at mid-day using a Sun Fleck Ceptometer. Total solar radiation was determined in an open field, and then an average value of solar energy remaining after interception by plants was determined by placing the sensor in three positions at ground level of crop canopy in each plot. The PAR intercepted was obtained by calculating the difference between total radiation and radiation remaining after interception by crop. Mature fresh pods were harvested from three inner rows (measuring 3 m^2) bi-weekly for about six weeks, beginning 80 days after emergence (DAE) for variety Ambassador and 90 DAE for variety Plum, and their weights determined. Fifteen pods were randomly taken from the pods harvested in each plot measuring 3 m^2 and their lengths determined using a ruler. Seeds from the 15 pods/plot were extracted and counted to determine the number of seeds per pod. Total fresh weight was determined by extracting all the seeds from all the pods harvested in each plot and measuring their weights. A hundred seeds were removed at random and weighed to determine 100-seed weight. At physiological maturity, number of pods per plant was determined from three plants initially selected randomly from the inner rows of a plot

3.4 Data analysis: Data collected were subjected to analysis of variance (ANOVA) using GenStat Discovery Edition 3 (VSN, 2008) at 5% level of significance. Treatment means were compared using Fisher's Least Significant Difference (LSD) method (Forthofer *et al*., 2007).

4 RESULTS

In the experimental site, the mean monthly rainfall ranged from 25-348.4 mm in the long rains and 36-75 mm in the short rains; while the number of rain days in a month varied from 5-17 in the long rains and 4 to about 14 in the short rains (Fig. 1). The long rains began during the first week of April during which the long rains crop was planted while the short rains began in early November during which the short rains crop was planted. The mean monthly temperature ranged from 16.3 to 19.6^{o}C while the relative humidity ranged from 50 to 72% (Fig. 2).

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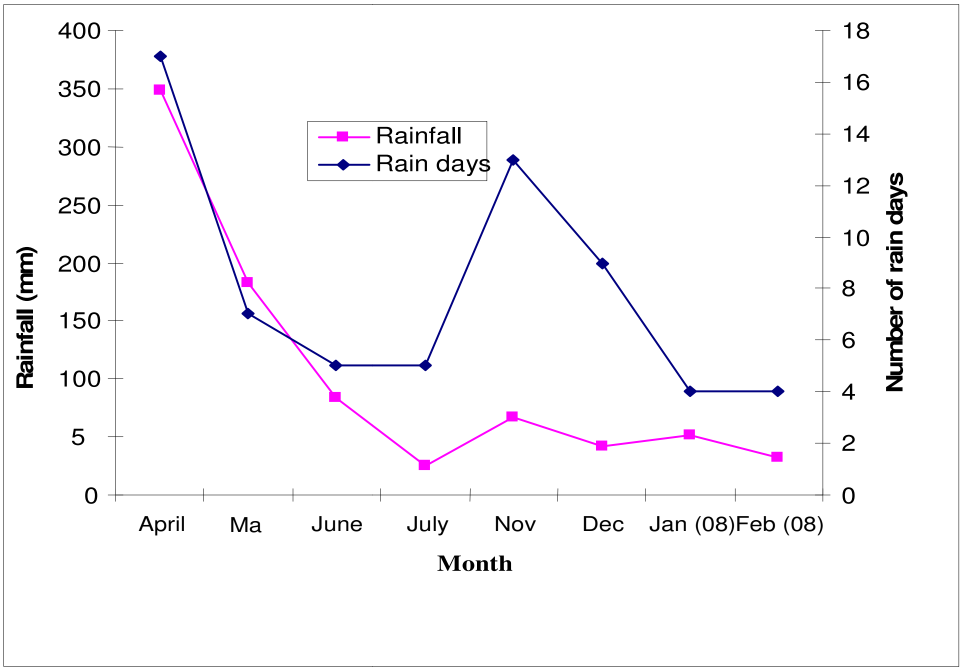

Figure 1: Mean monthly rainfall and rain days at University of Nairobi's Field Station in the long rains and short rain seasons of 2007

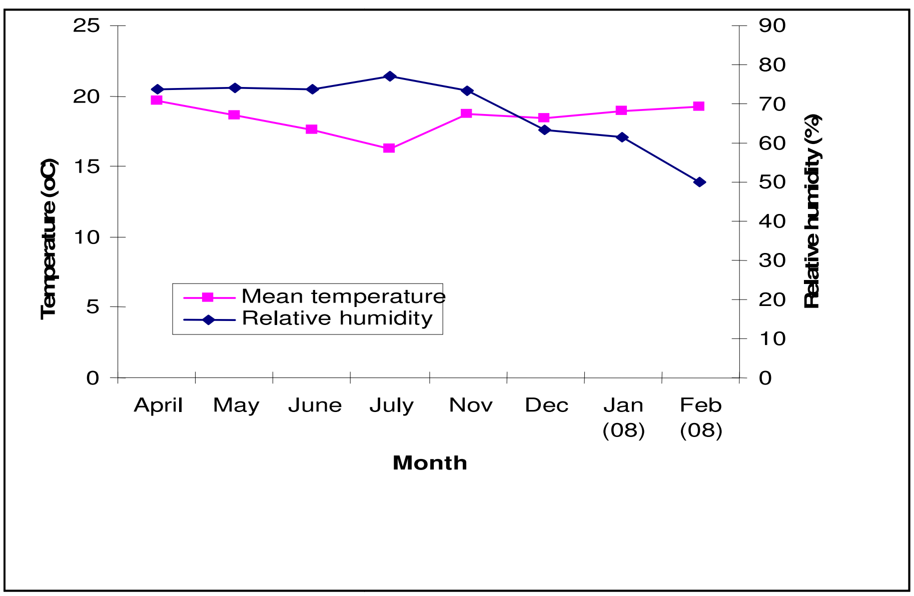

Figure 2: Mean monthly temperature and relative humidity at University of Nairobi's Field Station in the long and short rains season of 2007

Rhizobia inoculation and N fertilizer (30 and 60 kg N ha^{-1}) enhanced pea shoot biomass 7 WAE in the long rains season (Table 1). The interaction between nitrogen application and variety significantly (($P \leq 0.05$) affected shoot dry matter at 10 WAE (Table 1). Variety plum had a higher shoot biomass than Ambassador in control plots (0 kg N ha^{-1}) and plots supplied with 60 kg N ha^{-1}. Application of 30 and 60 kg N ha^{-1} increased shoot biomass in cultivars Ambassador and Plum, respectively. There were no significant treatment effects on pea shoot dry matter in the short rains season.

Table 1: Mean shoot dry matter (g/plant) of two garden pea (Ambassador and Plum) varieties inoculated with a commercial strain of *Rhizobium* or supplied with N fertilizer at 7 and 10 weeks after emergence (WAE) in the 2007 long and short rains

	Long rains (7WAE)	**Long rains (10 WAE)**			**Short rains (10 WAE)**		
Treatment (N)	**Mean**	**Ambassador**	**Plum**	**Mean**	**Ambassador**	**Plum**	**Mean**
Rhizobium	13.23_{x}	25.40^{a}_{y}	44.50^{a}_{y}	34.9^{a}	3.7	7.6	5.7^{a}
0 kg N ha^{-1}	5.28_{y}	18.10^{b}_{y}	57.40^{a}_{xy}	37.8^{a}	5.3	4.9	5.1^{a}
30 kg N ha^{-1}	13.13_{x}	55.90^{a}_{x}	41.40^{a}_{y}	48.7^{a}	5.0	7.3	6.2^{a}
60 kg N ha^{-1}	11.71_{x}	39.50^{b}_{xy}	83.20^{a}_{x}	61.3^{a}	8.2	7.5	7.9^{a}
Mean	10.84	34.7^{a}	56.6^{b}	45.7	5.6^{a}	6.8^{b}	6.2
LSD0.05 N	4.02		NS			NS	
LSD0.05							
Variety (V)			14.5			NS	
$LSD_{0.05}$ N x V			28.9			NS	

NS – not significant
Values followed by the same superscript letter (a, b) in a row or subscript letter (x, y) in a column are not significantly (P≤ 0.05) different, according to LSD test

Variety Plum had a higher leaf area index (LAI) than Ambassador at 0 kg N ha^{-1} and 60 kg N ha^{-1} (Table 2). Relative to the other treatments, application of 60 kg N ha^{-1} increased LAI in Plum. There were no treatments effects on LAI of Ambassador. Plum had a higher leaf area index than Ambassador. There was a significant nitrogen treatment effect on interception of PAR at 10 WAE in the long rains season only (Table 2). Pea in plots supplied with 60 kg N ha^{-1} intercepted 18.7% more PAR than untreated plots (0 kg N ha^{-1}). The rest of the treatments were not significantly different from the control. Generally, plants intercepted more PAR in the long rains than in the short rains season.

Table 2: Mean leaf area index and % photosynthetically active radiation (PAR) absorption, of two garden pea cultivars inoculated with a commercial strain of *Rhizobium* or supplied with nitrogen fertilizer, at 10 weeks after emergence in the 2007 long and short rain seasons

	Leaf Area Index				PAR	
Nitrogen treatments (N)	**Long rains**		**Short rains**		**Long rains**	**Short rains**
	Ambassador	Plum	Ambassador	Plum		
Rhizobia	1.60^{a}_{x}	3.59^{a}_{y}	0.98	1.15	83.60_{xy}	50.30^{x}
0 kg N ha^{-1}	1.31^{b}_{x}	3.95^{a}_{y}	0.98	1.1S1	74.10_{y}	49.70^{x}
30 kg N ha^{-1}	2.95^{a}_{x}	3.27^{a}_{y}	0.52	1.17	85.30_{xy}	49.40^{x}
60 kg N ha^{-1}	2.83^{b}_{x}	7.69^{a}_{x}	0.98	1.15	92.80_{x}	48.90^{x}
Mean	2.17^{a}	4.62^{b}	0.77^{a}	1.15^{b}	83.90	49.60
$LSD_{0.05}$ N	1.48		NS		12.04	NS
$LSD_{0.05}$ V	1.05		NS		-	-
$LSD_{0.05}$ N x V	2.10		NS		NS	NS

-not applicable.
Values followed by the same superscript letter (a, b) in a row or subscript letter (x, y) in a column are not significantly (P≤ 0.05) different, according to LSD test

There was a significant (P≤ 0.05) nitrogen treatment and variety interaction in determining the number of active nodules $plant^{-1}$ at 10 WAE (Table 3). Rhizobia inoculation enhanced number of active nodules in variety Plum in both seasons; and in variety Ambassador only in the short rains. Variety Plum had more active nodules than Ambassador in inoculated plots. Rhizobia inoculation significantly (P≤ 0.05) enhanced total nodule numbers $plant^{-1}$ only in the short rains 10 WAE (Table 3).

Table 3: Mean active and total nodule numbers plant^{-1} of two garden pea cultivars inoculated with a commercial strain of *Rhizobium* or supplied with nitrogen fertilizer, 10 weeks after emergence in the 2007 long and short rain seasons

Nitrogen treatments (N)	Number of active nodules				Total number of nodules	
	Long rains		Short rains		Long rains	Short rains
	Ambassador	Plum	Ambassador	Plum		
Rhizobia	4.70^{b}_{x}	29.30^{a}_{x}	1.25^{b}_{x}	5.00^{a}_{x}	19.85_{x}	5.38_{x}
0 kg N ha-1	8.30^{a}_{x}	10.70^{a}_{y}	0.00^{a}_{y}	0.00^{a}_{y}	18.50_{x}	0.88_{y}
30 kg N ha-1	8.70^{a}_{x}	2.70^{a}_{y}	0.00^{a}_{y}	0.00^{a}_{y}	7.80_{x}	0.62_{y}
60 kg N ha-1	2.70^{a}_{x}	9.70^{a}_{y}	0.00^{a}_{y}	0.00^{a}_{y}	16.70_{x}	0.50_{y}
Mean	6.10^{b}	13.10^{a}	0.30^{b}	1.25^{a}	15.00	1.84
$LSD_{0.05}$V	6.12		0.29		-	-
$LSD_{0.05}$ N					NS	1.32
$LSD_{0.05}$ NxV	12.24		0.50		NS	NS

- not applicable
Values followed by the same superscript letter (a, b) in a row or subscript letter (x, y) in a column are not significantly (P≤ 0.05) different, according to LSD test

Nitrogen treatment and variety interactions were significant (P≤ 0.05) for nodule dry matter at 10 WAE (Table 4). Variety Plum had higher nodule dry matter than Ambassador at 0 kg N ha^{-1} and in inoculated plots in both seasons; and at 60 kg N ha^{-1} in the long rains. Application of 30 and 60 kg N ha^{-1} depressed nodule dry matter in Plum and Ambassador, respectively, in the long rains, and in Plum alone in the short rains. Plum had 2.5 and 7 times more nodule biomass plant^{-1} than Ambassador in the long and short rains, respectively.

Table 4: Mean nodule dry matter (mg plant^{-1}) of two garden pea cultivars inoculated with a commercial strain of *Rhizobium* or supplied with nitrogen fertilizer, 10 weeks after emergence (WAE) in the 2007 long and short two rain seasons.

	Long rains			Short rains		
N treatments	Ambassador	Plum	Mean	Ambassador	Plum	Mean
Rhizobia	22.70^{b}_{yz}	103.30^{a}_{x}	63.00_{x}	0.33^{b}_{x}	3.33^{a}_{x}	1.83_{x}
0 kg N ha^{-1}	38.70^{b}_{xy}	94.90^{a}_{x}	66.80_{x}	0.34^{b}_{x}	3.33^{a}_{x}	1.83_{x}
30 kg N ha^{-1}	50.40^{a}_{x}	22.60^{b}_{z}	36.50_{y}	0.08^{a}_{x}	0.25^{a}_{y}	0.17_{y}
60 kg N ha^{-1}	3.30^{b}_{z}	64.00^{a}_{y}	33.70_{y}	0.25^{a}_{x}	0.17^{a}_{y}	0.21_{y}
Mean	28.80^{b}	71.20^{a}	50.00	0.25^{b}	1.77^{a}	1.01
$LSD_{0.05}$Variety (V)	10.92			0.27		
$LSD_{0.05}$ N	15.44			0.38		
$LSD_{0.05}$ NxV	21.84			0.54		

Values followed by the same superscript letter (a, b) in a row or subscript letter (x, y, z) in a column are not significantly (P≤ 0.05) different, according to LSD test

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Application of 30 and 60 kg N ha^{-1} significantly ($P \leq 0.05$) increased the number of pods plant^{-1} in both garden pea cultivars in the long rains only (Table 5), but N treatments had no effect on pod length, seeds pod^{-1} pod yield, 100-seed weight and seed yield in the two garden pea cultivars in both seasons (Table 5). Ambassador had longer pods and more seeds per pod than Plum in both seasons and in the long rains, respectively. Plum had a significantly higher number of pods plant^{-1} than Ambassador in both seasons (Table 6).

Table 5: Mean yield and yield components of two garden pea cultivars inoculated with a commercial strain of *Rhizobium* or supplied with nitrogen fertilizer, in the 2007 long rains season

N treatments	Pod length (cm)	Seeds pod^{-1}	Pods plant^{-1}	Pod yield (kg ha^{-1})	100-seed weight (g)	Seed yield (kg ha^{-1})
Rhizobia	6.76^{x}	5.21^{x}	21.72_{xy}	1887.00^{x}	35.37^{x}	522.00^{x}
0 kg N ha^{-1}	6.71^{x}	5.14^{x}	17.38_{y}	1661.00^{x}	36.86^{x}	421.00^{x}
30 kg N ha^{-1}	6.78^{x}	5.02^{x}	22.59_{x}	2077.00^{x}	34.01^{x}	516.00^{x}
60 kg N ha^{-1}	6.92^{x}	5.05^{x}	26.07_{x}	2292.00^{x}	33.79^{x}	609.00^{x}
Mean	6.79	5.12	21.92	1979	35.01	517
$LSD_{0.05}$ (N)	NS	NS	5.48	NS	NS	NS

Values followed by the same subscript letter (x or y) in a column are not significantly ($P \leq 0.05$) different according to Fisher's LSD test

Table 6: Mean yield and yield components of two garden pea cultivars inoculated with a commercial strain of rhizobia or supplied with nitrogen fertilizer, in the 2007 long rains season.

	Long rains			**Short rains**		
	Variety (V)			Variety (V)		
Parameter	Ambassador	Plum	$LSD_{0.05}$	Ambassador	Plum	$LSD_{0.05}$
Pod length (cm pod^{-1})	7.01^{a}	6.57^{b}	0.22	6.89^{a}	6.55^{b}	0.13
Seeds pod^{-1}	5.35^{a}	4.86^{b}	0.42	5.37^{a}	5.42^{a}	NS
Pods plant^{-1}	19.42^{b}	24.46^{a}	3.88	6.39^{b}	9.13^{a}	1.57
Total pod yield (kg ha^{-1})	1789^{a}	2170^{a}	NS	345.00^{a}	325.00^{a}	NS
100-seed weight (g)	35.74^{a}	34.27^{a}	NS	33.57^{a}	31.82^{a}	NS
Total seed yield (Kg ha^{-1})	484.00^{a}	550.00^{a}	NS	111.00^{a}	102.00^{a}	NS

NS – treatment effects were not significant
Values followed by different subscript letters (a, b) in a row are significantly ($P \leq 0.05$) different according to the LSD test

5 DISCUSSION

Nitrogen fertilizer increased shoot dry matter, leaf area index and PAR interception by garden pea. Similar increases in biomass accumulation in *Pisum sativum* L. have been reported (Atta *et al.*, 2004; Ballard *et al.*, 2004). The highest shoot dry matter in Ambassador was attained by application of 30 Kg N ha^{-1} while in Plum it was attained by application of 60 kg N ha^{-1}. Differential response of pea genotypes to N has been reported (Santalla *et al.*, 2001). Responses of pea to nitrogen fertilizer were not observed in the short rains perhaps due to low soil moisture. Nitrate-N moves by mass flow in soil, hence requires sufficient water for uptake (Jones, 2003).Inoculation of garden pea with rhizobia increased nodule numbers and weights as reported in previous studies (Habtemichial *et al.*, 2007). Inoculation improved the number of active nodules and nodule dry matter in Plum relative to Ambassador. Cultivar differences in nodulation have also been reported in pea (McKenzie *et al.*, 2001; Depret and Loguerre, 2008). Nodulation was suppressed in the short rains relative to the long rains possibly due to moisture stress caused by low rainfall. Moisture stress has been reported to reduce nodulation and nitrogen fixation (Ladrera *et al*., 2007). Application of 30 and 60 kg N ha^{-1} depressed nodule numbers in Plum and Ambassador, respectively. A nodule decrease of 70% has been reported in garden pea plots supplied with 100 kg N ha^{-1} (Chemining'wa and Vessey, 2006). However, application of 30 kg N ha^{-1} enhanced nodule biomass in variety Ambassador in this experiment. Similar nitrogen rates increased nodule biomass of *Pisum sativum* cv. Utrillo in Spain (Santalla *et al*., 2001). This suggests that Ambassador may require starter nitrogen to meet its nitrogen requirements before the onset of nitrogen fixation. Application of 30 and 60 kg N ha^{-1} gave the highest number of pods per plant in pea. Similar increases in pod numbers have been reported in garden pea (Uddin *et al*., 2000). The increased biomass accumulation and nodulation due to nitrogen fertilizer was not translated into increase in total fresh pod and seed yield. The site used for pea production in Kabete had soil nitrogen of 0.28 % which may have been sufficient for production of fresh pods and seeds in peas. Increases in fresh pod and seed yields of garden pea were reported in N-depleted soils (Abdul and Muhammad, 2006).

Nodulation observed in control plots indicates that native pea rhizobia in Kabete soils are compatible with Plum and Ambassador garden pea varieties. Large populations of indigenous *Rhizobium legumunosarum* bv. *viciae* can reduce yield benefits due to inoculation in faba bean and garden pea (Amanuel *et al*., 2000; Habtegebrial and Singh, 2006).. Although there were no pod and seed yield benefit, inoculation can be done in order to improve soil fertility in nitrogen depleted farms in Kenya. Research findings from one study showed that wheat grown in rotation with inoculated pea had better grain yield (Habtegebrial and Singh, 2006).

In conclusion, growth and nodulation responses to inoculation and nitrogen fertilization were dependent on the garden pea genotype, hence the need to investigate the differential responses of Plum and Ambassador. It is further suggested that a study be conducted to determine the effect of *Rhizobium* inoculation and nitrogen fertilization on a broad range of locally grown garden pea genotypes.

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