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# Effectiveness of indigenous pea rhizobia (*Rhizobium leguminosarum* bv. *viciae*) in cultivated soils of central Kenya

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### ABSTRACT

*Objective:* This paper reports on a study conducted to determine the effectiveness of pea rhizobia (*Rhizobium leguminosarum* bv. viciae) resident in Central Kenyan soils.

*Methodology and results:* Garden pea (*Pisum sativum* cv. Plum) grown in pots containing soils collected from 26 sites, with and without a history of pea cultivation, in central Kenya was inoculated with a commercial rhizobial strain, supplied with 74 mg N pot<sup>-1</sup>, or did not receive any treatment (control). Rhizobial inoculation enhanced pea nodule numbers in soils from some sites that had no history of pea cultivation. Nitrogen fertilizer depressed pea nodulation in soil samples from all sites. Most abundant active and total nodules (20 and 22 nodules plant <sup>-1</sup>) were recorded in sites with adequate soil N, high organic carbon and history of pea cultivation In many cases, plants in untreated soils had a high nodule number and accumulated more shoot biomass than plants growing in inoculated or nitrogen supplied soil. This confirmed the N<sub>2</sub> fixation efficiency of indigenous pea rhizobia strains. Plants that had poor nodule formation were those growing in soils from a site in Nyeri that was low in soil N and organic carbon.

*Conclusion and application:* Most soils in Central Kenya have abundant and efficient native strains of *pea rhizobia* irrespective of pea cultivation history. In some sites, indigenous rhizobia out-performed the commercial inoculant strain. Thus, it is advisable to screen indigenous pea rhizobia strains in the target sites for N<sub>2</sub> fixation efficiency with the objective of making more effective inoculants. Improvement in soil organic carbon in Central Kenya can enhance the benefits accruable from pea N<sub>2</sub> fixation. A similar study involving a broad range of pea genotypes and strains of *Rhizobium leguminosarum* bv. *viciae* is recommended.

#### INTRODUCTION

Central Kenya offers a favourable environment for garden pea (*Pisum sativum*) production. Garden pea exporters contract smallholder farmers or large-scale farms in this region (HCDA, 2008). Most smallholder farms are characterized by low soil nitrogen due to continuous cropping (Smaling, 1993; Voisin *et al.*, 2007). Nitrogen deficiency can be corrected by application of nitrogen fertilizer; however, excessive use of nitrogen fertilizer is potentially harmful to the environment. Global GAP rules which limit use of agrochemicals in food products exported to European Union could soon affect farmers using nitrogen fertilizers. It is reported that over 60% of Kenyan smallholder farmers had been locked out of export trade due to non-compliance to these rules (Graffham *et al.*, 2006). There is need therefore to look for alternative nitrogen sources for vegetable export

crops. Inoculation of pea with a commercial strain of *Rhizobium leguminosarum* bv. *viciae* is a good source of nitrogen, but the response of the crop to inoculation is attained in soils with low populations of indigenous garden pea rhizobia or with low soil nitrogen (Abdelgani *et al.*, 2002). Response of pea to inoculation may not be attained in soils with a history of pea cultivation as the population of rhizobia could be high (McKenzie *et al.*, 2001). Environmental factors like soil pH, moisture stress and salinity also affect populations of pea rhizobia in soil (Hansen, 1994). The presence and effectiveness of indigenous rhizobia nodulating garden pea in Central Kenyan soils has not been established. Much of the work on abundance of

#### MATERIALS AND METHODS

Experimental sites: Soil samples were taken from three farmers' fields each in four pea-growing areas of Central Kenya. The areas were: Nyeri (Kieni West), Kirinyaga (Inoi location), Limuru (Rironi location), Nyandarua South (Njabini location). Soil samples were taken from two different fields (with and without pea cultivation history) in each farm. Soil samples were also collected from two sites with and without pea growing history respectively, at University of Nairobi's Upper Kabete Campus Field Station. In each site, eight soil samples were randomly taken at a depth of 15 cm. bulked and mixed to obtain a composite sample weighing 14 kg. There were 26 composite samples in total and these were analyzed for soil macronutrients and pH using standard procedures of the National Agricultural Research Laboratories. A greenhouse experiment was then conducted at the University of Nairobi's Field Station between January and April 2008. Experimental design, treatments and crop husbandry : Garden pea cv. Plum was grown in 26 soils and subjected to either inoculation with rhizobia, application of 74 mg N pot<sup>-1</sup> and a control with neither rhizobia nor nitrogen application. The experiment was laid out in randomized complete block design with a factorial arrangement and treatments were replicated three times. The soils were placed in half kilogram pots. Two seeds of garden pea were sown in each pot at a depth of two inches. Rhizobium strain USDA 3474 obtained from the Soil Microbiology Laboratory of the University of Nairobi was applied as a seed dress. In rhizobia in Central Kenya has been done in common bean (*Phaseolus vulgaris* L), lima bean (*Phaseolus lunatus* L), cowpea (*Vigna unguiculata* L), green gram (*Vigna radiata* L), pigeon pea (*Cajanus cajan* L) and Lablab (*Lablab purpureus* L) (Anyango *et al.*, 1995; Karanja *et al.*, 2002; Chemining'wa *et al.*, 2011). One indirect method of determining the presence and abundance of indigenous rhizobia in a soil is by growing a host legume and then taking nodule counts (Somasegaran and Hoben, 1994). The objective of this study was to determine the effectiveness of indigenous garden pea nodulating rhizobia in Central Kenya soils.

order to avoid spread of rhizobia between the pots, inoculated pots received the treatments after the uninoculated pots. Each pot was placed onto a plastic plate. Nitrogen was applied in form of calcium ammonium nitrate in three splits, one at planting (starter nitrogen) and the others at two and four weeks after crop emergence respectively. Plants were watered every two days over a period of 60 days after sowing. Pots were kept weed-free by hand pulling beginning from two weeks after seed sowing. Pests and diseases were controlled by application of Bulldock® (Betacyfluthin) and Nimrod® (Bupirimate) at the rates of 50 ml and 60 ml per 100 litres of water, respectively, from two weeks after crop emergence to the end of cropping season.

**Data collection:** Nodule number, nodule dry matter, root dry matter and shoot dry matter were determined at 8 weeks after pea emergence. Two plants were carefully uprooted from each pot and roots washed in a bucket of clean water. Roots were separated from the shoots and nodules removed from roots of each plant and counted. Nodules with pink colour after dissection were considered to be actively fixing nitrogen. The roots, shoots and nodules were oven-dried at 50°C until constant weighs were attained. Collected data were subjected to analysis of variance (ANOVA) using GenStat Release 9.1. When treatment effects were significant, means were compared by Fisher's least significant difference test. Correlation analysis was done using SPSS 12.0.1 for windows.

#### RESULTS

Soil pH, organic carbon, nitrogen and phosphorous contents of the sampled sites are indicated in Table 1. Soil pH ranged from 4.52 (Nyeri #3) to 6.36 (Nyeri #2) while organic carbon varied between 1.8% (Nyeri #2) and 7.9% (Nyandarua #1). Nitrogen content varied from 0.2% (Nyeri #2) to 1.1% (Nyandarua #1) while

phosphorous content ranged from 29 ppm (Kabete) to 223 ppm (Nyeri # 1). There were significant ( $P \le 0.05$ ) site and nitrogen treatment interactions for pea active nodule numbers, total nodule numbers, nodule biomass, root biomass and shoot biomass (Tables 2-5).

**Table 1:** Soil characteristics at sampling sites

Site	Soil pH	Organic carbon (%)	Nitrogen (%)	Phosphorous (ppm)
Nyandarua #1	5.33	7.90	1.10	32
Nyandarua #2	5.12	3.10	0.45	62
Nyandarua #3	4.52	2.90	0.24	71
Nyeri #1	5.68	2.00	0.22	223
Nyeri #2	6.36	1.80	0.20	62
Nyeri #3	6.15	2.30	0.24	56
Kabete	5.22	2.40	0.28	29
Limuru #1	6.26	3.90	0.66	62
Limuru #2	5.69	3.10	0.60	32
Limuru #3	5.9	3.20	0.58	42
Kirinyaga #1	4.96	2.90	0.51	68
Kirinyaga #2	5.6	2.40	0.31	100
Kirinyaga #3	5.04	2.40	0.30	63

**Table 2:** Mean number of active and total nodules plant<sup>-1</sup> of garden pea grown in soils from different sites and supplied with nitrogen fertilizer or rhizobia inoculation, 8 weeks after emergence in a greenhouse experiment carried out in 2008

		Active nodules plant <sup>-1</sup>				Total nodule number plant <sup>-1</sup>				
		Nitrogen treatments (N)				Nitrogen treatments (N)				
	Pea	0 mg	74 mg			0 mg	74 mg			
Site (S)	history	pot <sup>-1</sup>	pot <sup>-1</sup>	Rhizobia	Means	pot <sup>-1</sup>	pot <sup>-1</sup>	Rhizobia	Means	
Kabete #1	Yes	8.50	0.00	5.60	4.70	11.00	0.00	9.93	6.98	
Kabete #1a	No	0.47	0.00	1.02	0.50	2.49	0.00	13.00	5.16	
Kirinyaga #1	Yes	6.43	0.00	4.67	3.70	12.23	0.20	26.00	12.81	
Kirinyaga #2	Yes	0.17	0.00	1.68	0.62	1.00	0.00	4.77	1.92	
Kirinyaga #3	Yes	2.40	0.00	0.89	1.10	5.20	0.00	3.87	3.02	
Kirinyaga #1a	No	0.00	0.00	0.67	0.22	0.00	0.00	1.67	0.56	
Kirinyaga #2a	No	0.83	0.00	8.00	2.94	1.50	0.00	13.00	4.83	
Kirinyaga #3a	No	13.00	0.00	5.43	6.14	19.00	0.00	10.33	9.78	
Limuru #1	Yes	3.00	0.00	2.67	1.89	5.70	0.00	10.63	5.44	
Limuru #2	Yes	20.00	0.00	6.10	8.70	20.00	0.00	9.90	9.97	
Limuru #3	Yes	12.00	0.00	2.43	4.81	15.57	0.00	12.10	9.22	
Limuru #1a	No	7.80	0.00	2.50	3.43	8.80	0.00	6.93	5.24	
Limuru #2a	No	5.23	0.00	0.98	2.07	8.57	0.00	3.77	4.11	
Limuru #3a	No	5.00	0.00	17.79	7.60	9.80	0.00	18.00	9.27	
Nyandarua #1	Yes	8.00	0.00	0.20	2.73	21.80	0.00	2.37	8.06	
Nyandarua #2	Yes	5.00	0.00	4.13	3.04	14.67	0.00	15.93	10.20	
Nyandarua #3	Yes	3.33	0.00	10.00	4.44	6.03	0.00	18.23	8.09	
Nyandarua#1a	No	10.00	0.00	6.30	5.43	16.00	0.00	7.43	7.81	
Nyandarua#2a	No	9.87	0.00	9.00	6.29	18.00	0.00	20.23	12.74	

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in cultivated soils	5								
Nyandarua#3a	No	8.33	0.00	1.33	3.22	14.37	0.00	2.80	5.72
Nyeri #1	Yes	2.00	0.00	1.32	1.11	4.00	0.00	4.80	2.93
Nyeri #2	Yes	7.00	0.00	11.00	6.00	8.83	0.00	18.00	8.94
Nyeri #3	Yes	2.00	0.00	5.33	2.44	2.00	0.00	49.90	17.30
Nyeri #1a	No	0.08	0.00	0.00	0.03	0.83	0.00	0.32	0.38
Nyeri #2a	No	0.93	0.00	2.10	1.01	12.00	0.00	5.53	5.84
Nyeri #3a	No	1.20	0.08	2.10	1.13	3.67	0.08	8.87	4.21
Mean		5.48	0.00	4.36	3.28	9.35	0.01	11.47	6.94
LSD <sub>0.05</sub> S			1.15				1.74		
LSD <sub>0.05</sub> N			0.38				0.58		
LSD <sub>0.05</sub> SxN			2.00				3.01		

Sites with 'Yes' pea history – garden pea grown within the last 3 years; sites with 'No' pea history – garden pea had not been grown before

**Table 3:** Mean nodule dry matter (mg plant<sup>-1</sup>) of garden pea grown in soils from different sites and supplied with

 nitrogen fertilizer or rhizobia inoculation, 8 weeks after emergence in a greenhouse experiment carried out in 2008

		Nitro	ogen treatments	(N)	
Site (S)	Pea cultivation history	0 mg pot <sup>-1</sup>	74 mg pot <sup>-1</sup>	Rhizobia	Mean
Kabete #1	Yes	17.40	0.00	7.52	8.31
Kabete #1a	No	9.30	0.00	13.60	7.63
Kirinyaga #1	Yes	12.23	0.67	43.27	18.72
Kirinyaga #2	Yes	0.33	0.00	11.80	4.04
Kirinyaga #3	Yes	5.07	0.00	9.53	4.87
Kirinyaga #1a	No	0.00	0.00	8.43	2.81
Kirinyaga #2a	No	2.33	0.00	20.30	7.54
Kirinyaga #3a	No	13.33	0.00	10.93	8.09
Limuru #1	Yes	33.90	0.00	32.47	22.12
Limuru #2	Yes	46.70	0.00	9.37	18.69
Limuru #3	Yes	29.50	0.00	9.05	12.85
Limuru #1a	No	4.88	0.00	10.40	5.09
Limuru #2a	No	10.00	0.00	2.83	4.28
Limuru #3a	No	8.00	0.00	33.00	13.67
Nyandarua #1	Yes	40.00	0.00	7.47	15.82
Nyandarua #2	Yes	18.20	0.00	22.00	13.40
Nyandarua #3	Yes	14.80	0.00	32.80	15.87
Nyandarua #1a	No	20.80	0.00	11.03	10.61
Nyandarua #2a	No	15.20	0.00	25.00	13.40
Nyandarua #3a	No	15.73	0.00	6.37	7.37
Nyeri #1	Yes	1.20	0.00	10.00	3.73
Nyeri #2	Yes	23.87	0.00	20.40	14.76
Nyeri #3	Yes	3.80	0.00	50.67	18.16
Nyeri #1a	No	2.00	0.00	0.67	0.89
Nyeri #2a	No	15.00	0.00	25.60	13.53
Nyeri #3a	No	7.09	0.33	11.68	6.37
Mean		14.26	0.04	17.16	10.49
LSD <sub>0.05</sub> S			3.00		
LSD <sub>0.05</sub> N			1.00		
LSD <sub>0.05</sub> SxN			5.19		

**Table 4:** Mean active nodules plant<sup>-1</sup> and nodule dry matter (mg plant<sup>-1</sup>) of garden pea grown in soils from different regions and supplied with nitrogen fertilizer or rhizobia inoculation, 8 weeks after emergence in a greenhouse experiment carried out in 2008

	Active n	odules			Nodule dry matter					
	Nitroger	n treatments	(N)		Nitrogen treatments (N)					
Region (R)	0 mg pot <sup>-1</sup>	74 mg pot <sup>-1</sup>	Rhizobia	Mean	0 mg pot <sup>-1</sup>	74 mg pot <sup>-1</sup>	Rhizobia	Mean		
Kabete	4.49	0.00	3.31	2.60	13.35	0.00	10.56	7.97		
Kirinyaga	3.81	0.00	3.18	2.33	5.55	0.11	17.38	7.68		
Limuru	8.84	0.00	5.41	4.75	22.16	0.00	16.19	12.78		
Nyandarua	7.42	0.00	5.16	4.19	20.79	0.00	17.45	12.75		
Nyeri	2.20	0.01	3.64	1.95	8.83	0.06	19.84	9.58		
Mean	5.35	0.00	4.14	3.16	14.14	0.03	16.28	10.15		
LSD R		1.15				3.00				
LSD N		0.38				1.00				
LSD RxN		2.00				5.19				

**Table 5:** Mean root dry matter (mg plant<sup>-1</sup>) and shoot dry matter (g plant<sup>-1</sup>) of garden pea grown in soils from different sites and supplied with nitrogen fertilizer or rhizobia inoculation, 8 weeks after emergence in a greenhouse experiment carried out in 2008

		Root dr	y matter		Shoot	Shoot dry matter				
		Nitroge	n treatmer	nts (N)		Nitrogen treatments (N)				
	Pea	0 mg	74 mg			0 mg	74 mg			
Site (S)	history	pot <sup>-1</sup>	pot <sup>-1</sup>	Rhizobia	Mean	pot-1	pot <sup>-1</sup>	Rhizobia	Mean	
Kabete #1	Yes	134.30	142.00	113.30	129.87	0.53	0.73	0.43	0.56	
Kabete #1a	No	244.00	141.70	219.30	201.67	0.80	1.45	0.71	0.99	
Kirinyaga #1	Yes	163.30	137.30	374.30	224.97	0.70	0.70	0.87	0.76	
Kirinyaga #2	Yes	274.30	125.30	165.20	188.27	0.38	0.81	0.45	0.55	
Kirinyaga #3	Yes	148.00	170.30	150.30	156.20	0.46	0.92	0.38	0.59	
Kirinyaga #1a	No	410.00	133.20	273.30	272.17	0.77	0.53	0.68	0.66	
Kirinyaga #2a	No	150.20	218.70	205.70	191.53	0.36	0.86	0.42	0.55	
Kirinyaga #3a	No	206.70	206.30	127.00	180.00	0.69	0.81	0.52	0.67	
Limuru #1	Yes	352.30	102.00	130.30	194.87	0.48	1.09	0.61	0.73	
Limuru #2	Yes	215.30	246.00	132.30	197.87	0.95	1.13	0.58	0.89	
Limuru #3	Yes	224.30	131.00	205.70	187.00	0.74	1.16	0.68	0.86	
Limuru #1a	No	237.00	110.30	189.00	178.77	1.13	0.90	0.60	0.88	
Limuru #2a	No	235.70	132.30	53.30	140.43	0.91	0.99	0.54	0.81	
Limuru #3a	No	169.30	82.80	114.30	122.13	0.84	0.98	0.46	0.76	
Nyandarua #1	Yes	193.30	86.70	180.30	153.43	0.68	0.96	0.53	0.72	
Nyandarua #2	Yes	232.70	90.80	180.70	168.07	0.89	0.67	0.61	0.72	
Nyandarua #3	Yes	234.70	101.60	237.00	191.10	0.88	0.91	0.82	0.87	
Nyandarua #1a	No	354.30	203.30	262.00	273.20	1.18	1.28	0.75	1.07	
Nyandarua #2a	No	133.20	117.70	195.00	148.63	0.67	1.05	0.93	0.88	
Nyandarua #3a	No	182.70	133.00	86.70	134.13	0.57	1.11	0.68	0.79	
Nyeri #1	Yes	96.70	71.70	113.50	93.97	0.60	0.46	0.59	0.55	
Nyeri #2	Yes	102.30	63.30	94.30	86.63	0.43	0.62	0.90	0.65	
Nyeri #3	Yes	73.70	107.70	126.70	102.70	0.37	0.88	0.45	0.57	
Nyeri #1a	No	107.70	102.90	62.30	90.97	0.65	1.05	0.69	0.80	
Nyeri #2a	No	156.70	86.70	152.30	131.90	0.55	0.70	0.48	0.58	
Nyeri #3a	No	101.00	71.70	77.70	83.47	0.61	0.55	0.51	0.56	

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Mean	197.45	127.55	162.38	162.46	0.69	0.90	0.61	0.73
LSD <sub>0.05</sub> S		67.74				0.21		
LSD <sub>0.05</sub> N		22.58				0.07		
LSD <sub>0.05</sub> SxN		117.34				0.36		

Pea plants grown in soil from Limuru #2 in which pea had been grown before had the highest active and total nodule numbers (Table 2). When inoculated peas were compared, soil from a site with no pea cultivation history (Limuru #3a) had plants with the highest number of active nodule numbers, while plants in soil from Nyeri #1a did not form active nodules and had a significantly lower pea total nodule numbers than most soils in both inoculated and untreated pots (Table 2). Kirinyaga #1a soil that had not been grown with pea did not form any nodules in uninoculated control pots. Rhizobia inoculation increased pea active nodule number and total nodule number in 5 and 10 soils, respectively, but decreased active nodule numbers in 8 soils. Application of nitrogen fertilizer reduced both active nodules and total nodule numbers (Table 2). Nyandarua #1 and Limuru #2, which had a history of pea cultivation, had higher total number of nodules than most sites with average nodule numbers of 21.8 and 20 nodules plant <sup>1</sup>, respectively (Table 2). Inoculated peas growing in soils from Nyeri #3 had the highest nodule biomass of 50 mg plant<sup>-1</sup> (Table 3). Rhizobia inoculation enhanced pea nodule biomass in 10 of 26 soils and depressed nodule biomass in 7 soil samples. Nitrogen fertilizer suppressed nodule biomass in most soil samples. Limuru and Nyandarua sites had significantly ( $P \le 0.05$ ) more pea active nodules in control pots than inoculated or nitrogen supplied pots (Table 4). In soils from all the regions, nitrogen fertilizer significantly (P≤ 0.05) suppressed active nodule formation. Under inoculated conditions, pea plants in Limuru soils had the highest number of active nodules, while those in Kabete and Kirinyaga soils had the least. Inoculation increased nodule biomass in pea plants in soils from Kirinyaga and Nyeri. Control pots with soils from Limuru had higher pea nodule biomass than the rest of the treatments. Pea plants in soils from regions with the

#### DISCUSSION

In control pots, three of the sites whose soils had the highest total and active nodule numbers of pea had a previous history of pea cultivation. In addition, untreated soils from Nyandarua, known for its commercial pea farming, had the highest number of active nodules and nodule biomass. In sites where pea is widely grown abundant nodulation has been reported highest active nodules had the highest nodule biomass. Rhizobia inoculation significantly increased root dry mass of pea plants in pots with soil samples from one site (Kirinyaga #1), but depressed root biomass in soil samples from 3 sites irrespective of their pea cultivation history (Table 5). Inoculated pea had higher root biomass than nitrogen fertilized pea in pots with soils from Kirinyaga #1, Kirinyaga #1a and Nyandarua #3. Compared to the control, nitrogen fertilizer decreased root biomass of pea in soils from 7 sites. Generally, untreated soils from Kirinyaga #1a had a higher pea root dry mass (410 mg plant <sup>-1</sup>) than most plants regardless of their treatment.

Rhizobia inoculation improved pea shoot dry matter only in pots with soils from site Nyeri #2. Inoculated pea plants in soils from 5 sites had reduced shoot biomass compared to the control, where 4 of the soils had no history of pea cultivation. All the plants in soils from Limuru sites without pea history had depressed shoot dry matter when inoculated. Pea plants supplied with nitrogen fertilizer had significantly enhanced shoot biomass in pots with soils from 10 of 26 sites, half of which had pea growing history. In control pots, pea plants in some soils, for example Nyandarua #1a and Limuru #1a, which had no pea cultivation history, had higher shoot biomass than plants grown in soils with pea cultivation history, irrespective of treatments applied. Linear regression analyses indicated that there were significant positive relationships between active nodule numbers and soil nitrogen (y = 7.473x + 2.470,  $R^2 = 0.346$ ,  $P \le 0.05$ ); total nodule numbers and soil nitrogen (y = 12.486x + 4.282, R<sup>2</sup> = 0.402, P $\leq 0.05$ ;), nodule dry matter and soil nitrogen (y = 25.928x +3.464,  $R^2 = 0.543$ ,  $P \le 0.05$ ), total nodule numbers and organic carbon (y = 2.028x + 3.460, R<sup>2</sup> = 0.390, P≤ 0.05), nodule dry matter and organic carbon (y = 3.804x + 3.020,  $R^2 = 0.430$ ,  $P \le 0.05$ ).

and this is translated into increased shoot weights ((Ballard *et al.*, 2004). Legumes have been reported to produce substrates that stimulate growth of rhizobia, and most rhizobial cells found in soil following homologous legume host plants possibly originate from nodules (Hirsch, 1996). Some sites that had no history of pea cultivation had higher nodule numbers. This may

be attributed to the previous cropping patterns that may have had specific host legumes such as lentil, vetch, faba bean and larthyrus, or rhizobia may have been transferred from pea cultivation sites through rain water (Ciafardini *et al.*, 1991; Hirsch, 1996).

Generally, untreated soils that had high levels of organic carbon had a high pea total nodule number. Regression analyses showed that there were significant positive relationships between nodule numbers and biomass with organic carbon. Olayinga et al. (1998) reported that organic amendments enhance nitrogen fixation in soil with low organic matter. Untreated soils from sites Limuru #2 and Nyandarua #1 that had peas with a high number of active and total nodules, respectively, had high soil nitrogen. In addition, the two sites had high nodule biomass. Regression analyses confirmed that there were significant positive relationships between nodule numbers and biomass with soil nitrogen. This appears to contradict observations by Ani et al. (2007) that high soil nitrogen inhibits nodule formation. However, nitrogen levels in the soils sampled were low. It has been observed that starter nitrogen is required in soil for use by legumes before nodules start fixing nitrogen (Hansen, 1994; Chemining'wa and Vessey, 2006). Rhizobia inoculation enhanced pea active nodule number in soils from 3 sites that had pea cultivation history, and also increased nodule weights in soils from 11 sites. Similar observations have been reported (Kiros et al., 2007; Huang and Erickson, 2007). This shows that commercial strains of rhizobia were more efficient in

#### CONCLUSIONS AND RECOMMENDATIONS

The study has demonstrated that most soils in Central Kenya contain abundant and effective *Rhizobium leguminosarum* bv. *viciae* strains irrespective of pea cultivation history. Soils from Nyandarua and Limuru had the most number of nodules hence rhizobial strains. In some cases, indigenous rhizobia appeared more efficient than the inoculant strain. Hence, the need to isolate indigenous *Rhizobium* strains in the target sites and screen them for N<sub>2</sub> fixation efficiency with a view to making more effective inoculants

#### REFERENCES

Abdelgani, M.E., Mohamed, S.S. and Osman, A.G. 2002. The use of Rhizobium inoculants for increasing productivity of cluster bean (*Cyamopsis tetragonoloba*) in desert-affected soils. In Challenges and imperatives for Biological Nitrogen Fixation Research and nitrogen fixation than the indigenous strains in these soils. Nodulation efficiency of commercial pea rhizobia over indigenous strains have been reported (Santalla et al., 2001). However, inoculated pea had depressed active nodules and nodule biomass in 8 sites and 7 sites respectively. These observations indicate that some soils in Central Kenya had indigenous strains of pea rhizobia that could be more efficient in nitrogen fixation than commercial ones. Similar reports have been recorded in lentils (McNeil et al, 2007). Native pea rhizobia in these soils could also have nodulating preference towards variety Plum used in the experiment. Host specificity of rhizobia in nodulation has been observed in pea (Depret and Laguerre, 2008). Nitrogen fertilizer depressed nodule formation in garden pea growing in soils from all sites. Similar observations have been made in garden pea (Voisin et al., 2002). This is because NIN gene, which plays a key role in nodule ontogenesis, is down regulated in the presence of high levels of nitrate or ammonium, hence inhibition of nodule formation (Ani et al., 2007). It was observed that rhizobia inoculation increased pea root and shoot biomass in soils from only one site. This shows that indigenous pea rhizobia in Central Kenya soils are abundant, competitive and efficient in nitrogen fixation, hence the high pea biomass accumulation in the control pots. Response of garden pea to rhizobia inoculation is attained in only soils that have low populations of indigenous rhizobia (Hafeez et al., 2000).

Nodulation, root and shoot biomass were positively correlated with high soil N and organic carbon. This means that improvement in soil fertility in central Kenya can enhance potential N-fixation benefits. It is recommended that a similar experiment involving a range of pea genotypes and strains of *Rhizobium leguminosarum* bv *viciae* be conducted. In addition, direct estimate of pea rhizobia population size should be determined using the most probable number method.

Application in Africa for the 21<sup>st</sup> Century. Ed. Karanja, N.K. and Kahindi, J.H.P. John Philips Africa Limited. 338 pages.

Adamu, A. 2001. Studies of rhizobium inoculation and fertilizer treatment on growth and production of faba bean (*Vicia faba*) in some 'yield-depleted'

and 'yield sustained' regions of Semien Shewa. Ethiopian Journal of Science 24: 197-211.

- Ani, B., Rogato, A., Enrica, D., Omrane, S. and Maurizio, C. 2007. Differential effects of combined N sources on early steps of the nod factor – dependent transduction pathway in *Lotus japonicus*. Molecular Plant-Microbe interactions. 20: 994-1003.
- Anyango, B., Wilson, K.J., Beynon, J.L and Giller, K.E. 1995. Diversity of rhizobia nodulating *Phaseolus vulgaris* L. in two Kenyan soils with contrasting pHs. Applied Environmental Microbiology 61: 4016-4021.
- Ardell, D. H and Curtis, A. R. 2006. Irrigated corn and soybean response to nitrogen under no-tillage in Northern Colorado. Agronomy Journal 98: 1367-1374.
- Ballard, R. A., Charman, N., McInnes, A. and Davidson, J. A. 2004. Size, symbiotic effectiveness and genetic diversity of field pea rhizobia (*Rhizobium leguminosarum* bv. viciae) populations in South Australian soils. Soil Biology and Biochemistry, 13: 1347-1355.
- Brady, N.C. and Weil, R.R. 2002. The Nature and Properties of Soils, 13<sup>th</sup> Edition. Pearson Education, New Jersey 07458. 960 pages.
- Chemining'wa, G.N., Theuri, S.M. and J. W. Muthomi. 2011. The abundance of indigenous rhizobia nodulating cowpea and common bean in central Kenyan soils. African Journal of Horticultural Science 5:92-97
- Ciafardini, G., Marinelli, G. and Missich, R. 1991. Soil biomass of *Bradyrhizobium japonicum* inoculated via irrigation water. Canadian Journal of Microbiology. 38: 584-587.
- Depret, G. And Loguerre, G. 2008. Plant phenology and genetic variability in root and nodule development strongly influence genetic structuring of *Rhizobium leguminosarum* biovar *viciae* populations nodulating pea. New Phytologist 179: 224-235
- Gregory, A., Dobrowolski, N., Morris, S. G., O'Connor,
  G. E. and Wallace, C. 1996. Nodulation of field-grown *Pisum sativum* and *Vicia faba*: Competitiveness of inoculant strains of *Rhizobium leguminosarum* bv. *viciae* determined by an indirect, competitive ELISA method. Soil Biology and Biochemistry 28: 247-255.

- Gan, Y.T., Jayakumar, P., Symons, S. and McDonald, C.L. 2008. Synergic effect of N and moisture on biochemical property of nodules and seed yield in chickpea. Australian Journal of Crop Science 1:11-22.
- Graffham, A., Karehu, E and McGregor, J. (2006). Impact of European on small-scale vegetable growers in Kenya. Fresh insights no.6, www. agrifoodstandards. org. Date accessed: 25-07-2007.
- Hafeez, H.Y., Shah, N.H., Malik, K.A. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. *viciae* strains for nitrogen fixation using nitrogen– 15 isotope dilution. Biology and Fertility of Soils 31: 65-69.
- Hansen, A.P. 1994. Symbiotic N<sub>2</sub> fixation of crop legumes. Achievements and perspectives. Margraf Verlag, Germany. 248 pages.
- HCDA, 2008. Kenya Horticultural Crops Development Authority export volume and value report. [Online]. Available from: www.hcda.or.ke. Date accessed: 16/01/09.
- Hirsch, P.R. 1996. Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytologist 133: 159-171
- Huang, H. C and Erickson, R. S. 2007. Effect of seed treatment with *Rhizobium leguminosarum* on *Pythium* damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. Journal of Phytopathology 155: 31–37.
- Jones, J.B. 2003. Agronomic Handbook Management of Crops, Soils and their Fertility. CRC press. 450 pages.
- Karanja, N., Wangaruro, S. and Anyango, B.M. 2002. Performance of indigenous bradyrhizobia strains isolated from Kenyan soils. In Challenges and imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21<sup>st</sup> Century. Ed. Karanja, N.K. and Kahindi, J.H.P. John Philips Africa Limited. Pages 338.
- Kiros, H.H., Bal, R.S and Jens, B. A. 2007. Wheat response to N<sub>2</sub> fixed by faba bean (*Vicia faba* L.) as affected by sulfur fertilization and rhizobial inoculation in semi-arid Northern Ethiopia. Plant Nutrition and Soil Science 170: 412-418
- McKenzie, R.H., Middleton, A.B., Solberg, E.D., DeMulder, N.F., Clayton, G.W and Bremer, E.

2001. Response of pea to rhizobia inoculation and starter nitrogen in Alberta. Canadian Journal of Plant Science 81: 637-643.

- McNeil, D.L and Materne, M. 2007. Lentil an ancient crop for modern times. Springer Netherlands. Pp 127-143.
- Miller, S.H., Elliot, R.M., Sullivan, J.T. and Ronson, C.W. 2007. Host-specific regulation of symbiotic nitrogen fixation in *Rhizobium leguminosarum* biovar *trifolii*. Microbiology 153: 3184-3195.
- Olayinga, A., Adetunji, A. and Adebayo, A. 1998. Effect of organic amendments on nodulation and nitrogen fixation by cowpea. Journal of plant nutrition 21:2455-2464.
- Paul. E.A and Clark, F.E. 1996. Soil Microbiology and Biochemistry. Academic Press, San Diego. 340 pages
- Rockefeller Foundation, 2002. Soil fertility degradation in sub- Saharan Africa: leveraging lasting solutions to a long- term problem. Conclusions from a workshop held at the Rockefeller Foundation Bellagio study and conference center. [Online]. Available at: www.ciat.cgiar.org/news/pdf/tsbf\_bellagio.pdf. Date accessed: 2/3/2007.
- Santalla, M., Amurrio, J. M and De Ron, A. M. 2001. Symbiotic interactions between *Rhizobium leguminosarum* strains and elite cultivars of *Pisum sativum* L. Journal of Agronomy and Crop Science 187: 59–68.
- Seguin, P., Craig., Sheaffer, C., Ehlke, N.J., Russelle, M.P and Graham, P. 2001. Nitrogen fertilization and rhizobial inoculation effects on kura clover growth. Agronomy Journal 93:1262-1268.
- Smaling, E.M.A. 1993. An agroecological framework for integrated nutrient management with special reference to Kenya. Doctoral thesis, Agricultural University, Wageningen, The Netherlands. 250 pages.
- Somasegaran, P. and Hoben, H.J. 1994. Handbook for Rhizobia. Springer – Verlag New York, Inc. 450 pages
- Sunripe, 2007. Sunripe company website. [Online]. Available from: www. sunripe.co.ke/farms.html -. Date accessed: 26-07-2007.
- Vocanson, A., Jean, R., Huber,t B. and Marie-Hélène, J. 2006. Effects of soil structure on pea (*Pisum sativum* L.) root development

according to sowing date and cultivar. Plant and Soil 281: 121-135

- Voisin, A.S., Bourion, V., Duc, G and Salon, C. 2007. Using an ecophysiological analysis to dissect genetic variability and to propose an ideotype for nitrogen nutrition in pea. Annals of Botany 100:1525-36.
- Voisin, A.S., Salon, C., Munier-Jolain, N.G. and Ney B. 2002. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). Plant and Soil, 242: 251-262.