ABUNDANCE OF INDIGENOUS RHIZOBIA NODULATING COWPEA AND COMMON BEAN IN CENTRAL KENYAN SOILS

Chemining'wa GN*, Theuri SWM and Muthomi JW
Department of Plant Science and Crop Protection, Faculty of Agriculture, University of Nairobi, P.O. Box 29053, Nairobi, Kenya
*Correspondence: umchemin@hotmail.com

Abstract

The abundance of indigenous soil rhizobia can determine whether a legume would respond to inoculation or not. This study was therefore conducted to determine the population size of indigenous soil rhizobia nodulating common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L.) in soils sampled from Kabete, Machakos, Nyeri and Kajiado sites. The most probable number technique was used. The treatments comprised common bean and cowpea rhizobia host traps and seven inoculant sources (five soils, commercial rhizobial inoculants and a control with no inoculation), laid out in a randomized complete block design with a factorial arrangement. The soils varied in chemical characteristics with Nyeri site having a low pH of 4 compared to pH of 6.4 at Kajiado site. Indigenous rhizobia nodulating cowpea ranged from 78.5 at Nyeri to more than 900 bacterial cells per gram of dry soil at Machakos and Kajiado. Common bean nodulating rhizobia were more than 900 bacterial cells per gram of dry soil in all the sites. The Nyeri site had the lowest rhizobia population for cowpea. In most sites, common bean produced significantly more nodules per plant than cowpea. A similar trend was observed with respect to nodule biomass though this was only significant with Kabete soils. Commercial inoculants produced more nodules for cowpea and more shoot biomass for both cowpea and common bean than inoculation with soil inocula. The results indicate that indigenous rhizobia are common in central Kenyan soils and vary from site. Further, commercial rhizobia strain appeared to be more effective in fixing N than indigenous rhizobia strains from the five field sites. Use of rhizobia inoculants in N-limited smallholder field conditions may be advisable to reduce reliance on inorganic N.

Key Words: Bean, Cowpea, Inoculant, Rhizobia, Soils.

Introduction

Biological nitrogen fixation involving host-specific symbiotic interactions between root nodule bacteria, collectively termed rhizobia, and legumes has received a lot of research attention because of the central role it plays in the maintenance of soil fertility (Sprent and Sprent, 1990). Legumes have the potential to contribute to soil N and increase yields of subsequent or associated non-nodulating crops through symbiotic nitrogen fixation (Brockwell et al., 1995).

One of the major strategies for enhancing and exploiting symbiotic nitrogen fixation by legumes in crop production systems is through rhizobial inoculation (Giller and Cadisch, 1995). Inoculation of legumes is especially critical when compatible rhizobia are absent, when population densities are low, or when native rhizobia not effective (Catroux et al., 2001; Brockwell et al., 1995; Giller and Cadisch, 1995). Soils lacking in rhizobia may occur in areas where indigenous related legumes are absent or where levels of pH, osmotic stress, temperatures and heavy metals are detrimental to rhizobial populations (Catroux et al., 2001; Hansen, 1994).

Host legumes can enrich their immediate soil environment with rhizobia through rhizosphere effects (Thies et al., 1995). Failure of inoculation to elicit response in legumes is a common phenomenon in Kenyan soils (Mureithi et al., 1998). This could be due to the presence of effective indigenous rhizobia or highly competitive

but inefficient indigenous strains that lock out the inoculant strains from occupying the nodules.

Rhizobial population sizes in agricultural soils vary widely. Estimates of bradyrhizobia population sizes of 63 soils from Africa, including four soils from Kenya, by Abaidoo et al. (2002) ranged from 0 to 104 cells g-1 soil. Population size of effective indigenous soil rhizobia can be used as a reliable index of whether a legume would respond to inoculation or not. Thies et al. (1991) showed that inoculation of eight leguminous crops with commercial strains increased the number of nodules per plant only soils containing 10 to 100 indigenous rhizobial cells g-1 soil. Preliminary studies conducted in 10 diverse sites in Kenya revealed that the population sizes of bradyrhizobia species (cowpea miscellany) were extremely low, a finding not supported by the good performance of the legumes (Mureithi et al., 1998). Hence, a follow-up study was conducted to determine the abundance of indigenous rhizobia nodulating cowpea and common bean in central Kenya soils.

Materials and Methods

Site Selection, Soil Sample Collection and Chemical Analysis

The study sites were selected based on agro-climatic conditions and prevalence of cowpea and common bean cultivation. Kajiado and Machakos represented the drier areas where both cowpea and common bean cultivation is prevalent while Nyeri and Kabete represented the wetter areas where common bean cultivation is prevalent but with little cowpea cultivation. The soil samples were collected from four cultivated field sites (Kajiado, Machakos, Nyeri, and Kabete) and one uncultivated field site at Kabete.

After clearing the surface debris, soils were sampled to a depth of 30 cm from five points (the center and at the four corners) within the selected fields. The soil samples from the five points were thoroughly mixed, bulked and sub-sampled. One sub-sample was used to determine the population of soil rhizobia while the other one was used for the chemical analyses. Soil samples for chemical analysis were air-dried and stored at room temperature until used. The bulked samples were analyzed at Kenya Agricultural Research Institute's National Agricultural Research Laboratories for soil pH, percent carbon, N, P, and electrical conductivity using established procedures.

Experimental Design and Treatments

The rhizobial populations in each soil were estimated using the most probable number technique as described by Somasegaran and Hoben (1994). Cowpea (*Vigna unguiculata*) was used as the trap host to check for the abundance of indigenous cross nodulating *Bradyrhizobium* spp which nodulate lablab (*Lablab purpureus*), cowpea, pigeon pea (*Cajanus cajan*), green gram (*Vigna radiata*) and lima bean (*Phaseolus lunatus*) while common bean (GLP-2) was used to determine the abundance of common bean specific *Rhizobium* spp.

The treatments comprised common bean (GLP-2) and cowpea (M66) "trap" host plants and seven inoculation sources: five soils at 7 dilution levels (from 10-1 then 2-1 to 2-6), commercial rhizobia inoculant and a control without inoculation. *Rhizobium* spp strain 2674 and *Bradyrhizobium* spp. strain 3456 obtained from the University of Nairobi's Soil Microbiology Laboratory were used to inoculate common bean and cowpea, respectively. The treatments were laid out in a randomized complete block design (RCBD) with a factorial arrangement and replicated three times. A total of 74 Leonard jars spaced 15 cm apart were arranged in each of the three blocks. The blocks were spaced 1 m apart.

The legume seeds were first pre-tested to determine the germination period and the information used to stagger pre-germination time of seeds to ensure synchronized germination among the legume species. After pre-testing, seeds were surface sterilized in 3% sodium hypochlorite solution for 3 –5 minutes, then rinsed in 95% alcohol to remove waxy material on the

surface and trapped air, followed by rinsing in at least 6 changes of sterile water. The seeds were then soaked in water in a refrigerator for four hours to imbibe water then placed in a germination chamber for pre-germination. Three to four healthy seedlings were planted in every Leonard jar then inoculated with the diluted soil samples and later thinned to 2 plants per jar seven days after emergence.

The whole soil inocula were diluted to 10-1 by suspending about 20 g of each soil sample in 80 ml of sterile water and then shaking for 15 to 20 minutes with a wrist shaker. Other dilution series ranging from 2-1 to 2-6 were prepared from the lowest dilution of 10-1. An aliquot of 1 ml of diluent was used to inoculate each seedling in the Leonard jars. Application and regular checking of levels of nitrogen–free nutrient solution was done on daily basis to ensure that the seedlings were adequately moistened. Sterilized sand in Leonard jars was used as the growth medium.The plants in the Leonard jars were scored for the presence or absence of nodules 28 days after inoculation. The presence of a single nodule in a Leonard jar was considered a positive score. The most probable number technique was used to determine rhizobia cells per gram of dry soil (Somasegaran and Hoben, 1994). The roots were carefully washed with tap water to remove sand, and then the attached foam material and the wick carefully removed taking care not to destroy the roots and nodules. The nodules were then counted. The shoots were separated from roots and separately oven-dried at 600C for 48 hours and their respective biomass determined.

Data Analysis

Analysis of variance (ANOVA) was performed on the number of nodules, shoot dry matter and root dry matter using General Statistics (GENSTAT®) package (Genstat, 1995). When the F test was significant, treatment means of the parameters were separated by the least significant difference (LSD) test at 5% probability level (Steel and Torrie, 1987).

Results

The sites from which the soils were collected varied in pH, organic C, N, P, and EC (Table 1). The Nyeri site had a very low pH level compared to the other sites. The Machakos site had a low N and organic carbon but high in P compared to the other sites.

All the soil samples collected from the five field sites contained common bean and cowpea nodulating indigenous rhizobia (Table 2). The population size of indigenous rhizobia in the field sites varied from 78.5 to more than 900 bacterial cells per gram of dry soil (Table 2). When common bean was used as the trap host, all the field sites had more than 900 bacterial cells per gram of dry soil g-1 soil, while when cowpea was used only soils from Kajiado and Machakos field sites produced a similar number. Soil from Nyeri produced the lowest number of bacterial cell per g-1 soil for cowpea compared to the other soils. Cultivated Kabete field site recorded a higher population size of indigenous cowpea rhizobia than the uncultivated Kabete field site.

The interactive effects of the field sites and the legume species on nodule count per plant were highly significant ($P \leq 0.01$). Generally, common bean produced higher number of nodules per plant than cowpea in most of the sites (Table 3). The highest nodule count per plant for common bean was observed when soils from cultivated Kabete and Kajiado soils were used, while the least was observed with Nyeri and Machakos soils. Cowpea produced significantly ($P \leq 0.05$) the highest number of nodules per plant when Machakos soils were used compared to soils from the other sites. Use of commercial inoculants significantly increased the number of nodules for cowpea compared to soils from the five sites (Table 3). For common bean, commercial inoculants were only significantly effective when compared to soils from Nyeri.

The interactive effects of the field sites and the legume species on shoot dry matter per plant were highly significant ($P \leq 0.01$).

Common bean produced significantly higher shoot biomass across the field sites except for the soils from Nyeri (Table 4). There were, however, no significant differences in shoot biomass among the sites for both crops. Inoculation with commercial inoculants significantly ($P \leq 0.05$) increased shoot dry matter per plant for both crops when compared to soils from all the five sites.

Table 1: Chemical characteristics of the five field sites in Central Kenya

Soil Properties	**Kabete cultivated**	**Kabete uncultivated**	**Kajiado**	**Nyeri**	**Machakos**
pH H2O	6.20	6.20	6.40	4.00	6.80
% C	3.50	4.70	4.70	3.10	1.70
% N	0.38	0.40	0.50	0.28	0.21
EC dsm-1	0.30	0.36	0.32	0.12	0.41
P (ppm)	25.0	20.0	30.5	3.50	42.2

Table 2: Number of rhizobial bacteria cells g-1 soil in five field sites in central Kenya.

Trap host	**Kabete (cultivated)**	**Kabete (uncultivated)**	**Kajiado**	**Nyeri**	**Machakos**
Common bean	$>9.0 \times 10^2$	$>9.0 \times 10^2$	$>9.0 \times 10^2$	$>9.0 \times 10^2$	>9.0 X 102
Cowpea	311.6	207.9	>900.3	78.5	>900.3

Table 3: Mean number of nodules per plant of common bean and cowpea inoculated with soils from five field sites in central Kenya and a commercial rhizobia inoculant at 28 days after inoculation

Trap host	**Number of Nodules**		
	Common bean	Cowpea	Mean
Kabete - Cultivated	36.4	7.3	21.8
Kabete - uncultivated	28	3	15.5
Kajiado	44.8	5.7	25.3
Machakos	19.6	22.6	21.1
Nyeri	7.3	0.6	3.9
Rhizobia inoculated	15.3	38	26.7
Un-inoculated	0	0	0
Mean	21.6	11	16.3

Table 4: Mean shoot dry matter (g) per plant of common bean and cowpea inoculated with the soils collected from five field sites and a commercial rhizobia inoculant at 28 days after inoculation

Trap host	**Number of Nodules**		
	Common bean	Cowpea	Mean
Kabete - Cultivated	0.57	0.34	0.47
Kabete - uncultivated	0.57	0.36	0.47
Kajiado	0.68	0.37	0.53
Machakos	0.69	0.37	0.53
Nyeri	0.45	0.34	0.39
Rhizobia inoculated	1.27	0.68	0.98
Mean	0.71	0.41	

Discussion

The indigenous populations of rhizobia (bradyrhizobia) nodulating cowpea ranged from 78.5 (at Nyeri) to more than 900 bacterial cells per gram of dry soil (at Kajiado and Machakos), while common bean nodulating rhizobia were more than 900 bacterial cells per gram of dry soil in each of the sites. These observations provide evidence that indigenous common bean rhizobia (*Rhizobium* spp) and cowpea rhizobia (*Bradyrhizobium* spp) are widespread in central Kenya. The high population levels of common bean and cowpea nodulating ingenious rhizobia in the field sites could be attributed to the legumes' widespread integration in the cropping system in Kenya (Gethi et al., 1997; Wortmann and Allen, 1994). This finding is in agreement with Mahler and Wollum (1981, 1982) who reported an increase in soil indigenous rhizobia population from 0.1% (in absence of legume) to 8-9% of the total aerobic bacteria when legumes were cultivated in the field.

Variation among the field sites in population sizes of bradyrhizobia bacteria (i.e. 78.5 to >900 cells per gram of dry soil) observed in this study is a common phenomenon. This can be attributed to differences in levels of soil pH, plant nutrients, soil type, soil moisture, temperature and crop/soil management, among other factors (Giller, 2001; Hansen, 1994). The results are in agreement with the findings of several authors. Abaidoo et al. (2002) reported population sizes ranging from 0 to 104 cells g-1 soil in 63 soils from Africa including soils from Kenya. Similar population size variations have been observed (Singleton et al.1992; Thies et al., 1991; Woomer et al., 1988). The low population of rhizobia and legume nodulation noted with the Nyeri soil could be attributed to its low pH (4.00) and P-deficiency (3.5 ppm) that have been shown to adversely affect both survival of rhizobia and nodulation process in legumes (Graham. 1992). Nodules are reported to be stronger sinks for P than roots, shoots and even young mature leaves (Graham, 1992).

In most sites, common bean produced more nodules per plant than cowpea. This could be attributed to genetic differences between the varieties of the two species. Nodulation capacity is known to vary between and within legume species (Hansen, 1994). Relative to soil inoculations, inoculation with commercial inoculant improved nodulation for cowpea and shoot biomass for both cowpea and common bean. This finding suggests that the commercial strains were more efficient in fixing N than indigenous rhizobia.

Conclusions and Recommendations

The study results have shown that native rhizobia that nodulate common bean and cowpea are widespread in central Kenyan soils and vary in population size from site to site. Low pH and deficient levels of P appear to adversely affect rhizobia cell numbers and nodulation. The commercial inoculant was more effective in terms of shoot biomass improvement than the soil inoculants. This calls for promotion of the use of rhizobia inoculants in N-limited field conditions. Use of rhizobia inoculants may reduce reliance on inorganic N that is expensive to most smallholder farmers.

References

Abaidoo RC, Keyser, HH, Singleton, PW. 2002. Population and symbiotic characteristics of indigenous Bradyrhizobium spp. that nodulate TgX soybean genotypes in Africa. Paper presented at Ninth Congress of the African Association for Biological Nitrogen Fixation, held in Nairobi, Kenya, 25-29 September 2000. Pages 167-188.

Brockwell J, Bottomley PJ, Thies JE. 1995. Manipulation of rhizobium microflora for improving legume productivity and soil fertility: A critical assessment. Plant and Soil 174, 143-180.

Catroux G, Hartmann A, Revelin C. 2001. Trends in rhizobial inoculant production and use. Plant and Soil 230, 21-30.

Genstat 1995. Genstat statistical software. Lawes Agricultural Trust, Rothamstead Experimental Station.

Gethi M, Muriithi FM, Macharia N, Njoroge K. 1997. Maize/bean intercropping system in medium potential area of Kenya. Farmer's Practice and Research Challenges. African Crop Science Conference Proceedings 3, 756-770

Giller KE. 2001. Nitrogen fixation in the tropical cropping systems. Cabi Publishing, Wallingford, UK. Pp 400.

Giller KE, Cadisch G. 1995. Future benefits from biological nitrogen fixation: An ecological approach to agriculture. Plant and Soil: 174, 255-277

Graham PH. 1992. Stress tolerance in Rhizobium and Bradyrhizobium, and nodulation under adverse soil conditions. Canadian Journal of Microbiology 38, 475-484.

Hansen AP. 1994. Symbiotic N2 fixation of crop legumes: Achievements and perspectives. Centre for Agriculture in the Tropics and Subtropics, University of Hohenheim, Germany. Margraf Verlag, Weikersheim, Germany.

Mahler RL, Wollum II AG. 1981. The influence of water potential and soil texture on the survival of Rhizobium japonicum and Rhizobium leguminosarum isolates in the soil. Soil Science Society American Journal 45, 761-766.

Mahler RL, Wollum AG. 1982. Seasonal fluctuation of Rhizobium japonicum under a variety of field conditions in North Carolina. Soil Science 134, 317-324.

Mureithi JG, Maobe SN, Dyck E, Gachene CKK, Gitari N, Kirungu B, Muli BM, Ojiem J, Saha HM, Tana P. 1998. Screening of legume germplasm in Kenya: Effect of rhizobia inoculation on performance of best-bet legumes. In: Shayo-Ngowi, AJ., Ley, G. and Rwehumbiza,, FBR. (Eds). Soil Science Society of East Africa proceedings of the 16th Conference, held 13-19 December 1998, Tanga, Tanzania.

Singleton PW, Bohlool BB, Nakao PL. (1992) Legume response to rhizobial inoculation in the tropics: myths and realities. In: Myths and Science of Soils of the Tropics. pp. 135-155. Soil Science and Society of America and American Society of Agronomy. Special publication No. 29.

Somasegaran P, Hoben HJ. 1994. Handbook for Rhizobia. Springer laboratory.

Sprent JI, Sprent P. 1990. Nitrogen fixing organisms. Pure and applied aspects. Chapman and Hall. London

Steel GDR, Torrie JH. 1987. Principles and Procedures of Statistics. A Biometrical Approach. 2nd Edition, McGraw-Hill Book Company, London, UK.

Thies JE, Singleton PW, Bohlool BB. 1991. Influence of the site of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Applied Environmental Microbiology 57, 19-28.

Thies JE, Woomer PL, Singleton PW. 1995. The enrichment of Bradyrhizobium spp population in soil due to cropping of homologous host legume. Soil Biology and Biochemistry 27, 633-636.

Woomer P, Singleton PW, Bohlool BB. 1988. Ecological indicators of native rhizobia in tropical soils. Applied Environmental Microbiology 54, 1112-1116.

Wortmann CS, Allen DJ. 1994. African bean production environments: their definition, characteristic and constraints. Network on Bean Research in Africa, occasional series No. 11 Dar es Salaam, Tanzania.