Research Journal of Agriculture and Biological Sciences, 4(6): 810-818, 2008

Nodulation *in Situ* of Common Bean (*Phaseolus vulgaris* L.) And Field Outcome of an Elite Symbiotic Association in Senegal

[1,2]Adama Diouf, [1]Tahir Abdoulaye Diop and [2]Mamadou Gueye

[1]Laboratoire de Biotechnologie des Champignons, Département De Biologie Végétale, Faculté des Sciences et Techniques UCAD B.P 5005, Dakar, Sénégal
[2]MIRCEN/Laboratoire Commun De Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, B.P. 1386, Dakar, Sénégal.

Abstract: The investigation of common bean rhizobial indigenous population in Senegal indicated a low rate of nodulation in field. However fifty eight rhizobial strains were isolated from root nodules of common bean (*Phaseolus vulgaris*) growing in soil sampled from various Senegalese localities. An elite symbiotic association *Rhizobium* strain ISRA 355 x common bean variety Bronco was selected from the greenhouse experiment conducted at Bel Air experimental station. This efficient association was used in an experimental field carried out at this station to assess effect of rhizobial inoculation on common bean. Inoculation with *Rhizobium* strain ISRA 355 and nitrogen fertilizer application (0, 20 or 80 kg N/ha) were significant on shoot and root dry weights mainly. However, pod yields and nodules dry weight were significantly higher in inoculated and nitrogen (20 kg N/ha) treated plots than in uninoculated: +77% and +300% respectively. Pod yield was highly improved (+77%) by the interaction between inoculation with ISRA 355 and nitrogen fertilizer at 20 kg N/ha. Whatever the nitrogen rate was, inoculation of common bean increased the acetylene reduction activity (+156%) in comparison to the uninoculated plants. Similarly in inoculated treatments, the relative abundance of ureide-N in plants treated with 20 kg N/ha was higher than that of plants unfertilized or amended with 80 kg N/ha for +10% and +16% respectively.

Key words: Inoculation, nitrogen fixation, nodulation, *Phaseolus vulgaris*, *Rhizobium*, Senegal.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important legume crop worldwide originated in the Americas[12] and is the more cultivated species of the genus *Phaseolus* in Latin America. Common bean occupied also an important place in East and South African agricultural system[21,11]. In semi-arid and dry sub-humid Savanna of West African region, bean *Phaseolus* is not widely grown or known in rural zones. The crop is managed by some farmers in few gardens around houses where microclimate is favorable. In Senegal, common bean is intensively cultivated in the cold and dry period out off the rainy season in an irrigated cropping system in Niayes agro-ecological zone from Dakar to Saint Louis along the costal area. In this zone soils are sandy and low on mineral nutrients. Since consumption of bean is limited in urban area, the main part of the production (83%) is assigned to exportation in Europe. It constitutes a profitable cash crop for farmers. Thereby farmers' management of culture is an intensive monoculture system. In this cropping system high amount of nitrogen fertilizer is used for increasing bean productivity and this become a main factor for groundwater pollution which is enriched for nitrates by soil leaching. This water table pollution constitutes health insecurity for the lakeside residents.

The benefits of biological nitrogen fixation (BNF) must be intensively explored to increase yield at low cost. The BNF can constitute an alternative to the use of expensive chemical nitrogen fertilizer or at less permits its reduction in agricultural system. Bean leguminous plants are classified in the ureide plant group[18] for which nitrogen fixed by symbiotic rhizobia is incorporated in the ureide molecules.

This work aims mainly to enhance Senegalese common bean productivity with rhizobial bio-fertilizing inoculums in order to minimize groundwater pollution. In this way we proposed to estimate indigenous rhizobial populations and selected an elite *Rhizobium* x bean cultivar to determine effect of inoculums on bean growth and yield and nitrogen fixed in field.

MATERIAL AND METHODS

Soils and plants sampling: Soils were sampled in fifty nine localities ranged in five different agro-ecological zones in Senegal: Niayes, Fleuve, Sylvopastorale, Bassin Arachidier and Sénégal Oriental-Casamance. Soils were sampled from topsoil (0 to 20 cm) and stored at 4°C in the laboratory for rhizobium trapping

Corresponding Author: Dr Adama Diouf, Laboratoire De Biotechnologie Des Champignons, Département De Biologie Végétale, Faculté Des Sciences et Techniques Ucad B.p 5005, Dakar, Sénégal
E-mail: Adiouf97@yahoo.com, Tel:+221 689 85 87, Fax:+221 33864 66 58.

in greenhouse culture. Soil types collected were different from their texture, color and previous culture or predominant vegetation in the locality. Main experiments were carried out at Bel-Air experimental station. The soil was a sandy type (94% of sand). The pH of the soil was 7.0 with 0.025% nitrogen, and 37 ppm available phosphorus. Common bean plants were sampled in farmers' fields in Niayes zone (31 plants) where common bean was cultivated intensively with high nitrogen fertilizer applied and in Fleuve (12 plants) where bean crops were not largely farmed. In addition, some undestroyed plants were also operated in farmers' fields for nodulation status in these two agro-ecological zones.

Estimation of native *Rhizobium* infecting bean in Senegalese soils: The plant infection count, also know as most-probable-number (MPN) counts was used to determine the number of viable and infective rhizobia. Ten grams of soil sampled were diluted in aseptic saline solution (0.8%NaCl). One milliliter of a fivefold dilution was used to inoculate a common bean seedling adequately grown in Gibson tube[13] on four replicates. Positive and negative nodulation of growth unit were recorded for all dilutions and the number of rhizobia was estimated referred to MPN table[4].

Greenhouse experiment: A greenhouse experiment was carried out at Dakar, in Bel-Air station to select an elite symbiotic association between collected rhizobial strains and common bean varieties. The four bean cultivars (Bronco, Delinel, Rocdor and Victoire) were grown in pots (one seedling/pot) contained 1.2 kg of non sterile Bel Air soil. One milliliter of liquid inoculums (10^8 *Rhizobium*/ml) of these ten rhizobial strains (ISRA 350, 351, 353, 354, 355, 356, 357, 361, 362 and 363) were used separately to inoculate seeds at the sowing stage. *Rhizobium* strain x common bean variety associations were in a randomized completed bloc design with four replicates. Plants were regularly watered with unfertilized tap water. At 30 days after sowing, all plants were harvested. Nitrogenase activity of rhizobial strains was assessed using the technique described by Hardy *et al.*[16] to determine acetylene reduction activity (ARA). Plant shoot fresh weigh (SFW) and dry weigh (SDW) were measured.

Field experiment: A field experiment was conducted at Bel Air in the same station than greenhouse experiment. The seeds of common bean varieties were surface sterilized by immersion in 95% ethanol for 3 min and 0.1% $HgCl_2$ for 3 min and then washed with sterile water. After washing, seeds were hand sown in a randomized complete factorial design with four replicates. The size of each plot was 1.50 x 2.55 m with 15 cm and 40 cm within and between rows respectively. The main factor was the inoculation: seeds inoculated with peat slurry containing 10^8 rhizobia/g and no inoculated seeds. The rhizobium strain ISRA 355 used was selected for it high N_2 fixing potential in association with the variety Bronco from greenhouse experiment. The sub-factor was the nitrogen fertilizer applied as ammonium sulfate with tree levels: 0, 20 and 80 kg/ha. To all plots, a basal fertilizer was added consisting of 60 kg/ha as a triple superphosphate and 120 kg K as KCl. At 50 days after sowing, plants were harvested in each sub-plot from the two intern rows. The harvested plants were separated into different plant parts. The ARA was evaluated. Plant shoots, pods, roots and nodules dry weights were recorded.

Analytical procedure: From both experiments, total nitrogen of harvested plant shoot was calculated from N contents (N %) determined by micro Kjeldahl technique described by Neil[27].

The relative abundance of ureide-N (Ur-N%) in xylem sap of shoot tissue extracted from stems before antepenultimate leave was calculated from the molar concentration of ureides, amino-acids, nitrates and amides. Concentration of ureides (allantoin and allantoic acid) in xylem sap were measured colorimetrically as phenylhydrazone derivative of glyoxylate[39,3]. Nitrates in xylem sap were measured by the sulfanilamide technique[33]. The concentration of amides (asparagine plus glutamine) was assayed using the technique proposed by Boddey *et al.*[3] based on the method of hydrolyze of amides in ammoniac[25]. The total content amino acids of sap were determined colorimetrically by the ninhydrin method[31]. Assuming that ureides, amides, amino acids and nitrates contain 4, 2, 1 and 1 atom N per molecule, respectively, the relative abundance of ureide-N (Ur-N %) in xylem sap was calculated as:

$$\text{Ur-N (\%)} = 400 \times [\text{ureides}] / ([4 \times \text{ureides}]+[2 \times \text{amides}]+[\text{amino acids}]+[\text{nitrates}])$$

Data from both experiments were statistically analysed and compared using Newman and Keuls test when the F-test from the analysis of variance (ANOVA) was significant at P = 0.05.

RESULTS AND DISCUSSION

Results: Nodulation survey of *Phaseolus vulgaris* in Senegal: Few nodule numbers were harvested from common bean plants sampled from farmers' fields at three localities named Keur Moussa, Camberène and Thialène in the Niayes zone. On the other hand, in Fleuve zone, inefficient nodules were observed on plants sampled from two farmers' fields. Nodules were only efficient in Niayes zone. On the whole plants sampled on the field, three strains were only isolated from nodules harvested *in situ* in a trial experiment conducted at Camberène by the Professional Formation Center for Horticulture in collaboration with *Tropica Sem*, a locale seeds trading society. The majority of *Rhizobium* germplasm collected was isolated by trapping system from soil samples. From the 59 investigated sites where soils were sampled, 58 *Rhizobium* strains were isolated in 25 localities differently distributed in the five agro-ecological areas (Table 1). Moreover some *Rhizobium* strains were isolated in the same site. The number of *Rhizobium* strains isolated varied in the localities.

Table 1: Number of sites investigated, sites of isolation and *Rhizobium* strains isolated from these sites in Senegalese soils.

Agro-écologique areaa	Number of investigated sites	Number of sites with positive nodulation	Number of strains isolated
Niayes	10	4	6
Fleuve	12	7	17
Sylvopastorale	09	3	9
Bassin arachidier	21	10	23
S. O. – Casamance	07	1	3
Total	59	25	58

a S.O.: Sénégal Oriental.

Estimation of native *Rhizobium* infecting bean in Senegalese soils: Population of indigenous *Rhizobium* strains was estimated by Most Probable Numbers (MPN) method from soils sampled at the different agro-ecological areas. This study showed a few *Rhizobium* strains infective on common bean *P. vulgaris* in Senegalese soils. The lowest number recorded in Velingara soils located in Senegal Oriental- Casamance agro-ecological zone and the highest number found at Camberène in Niayes zones. The number of *Rhizobium* ranged in average from 16 to 120 cells/g soil in total agro-ecological zones. At Bel Air site, in the Niayes, zone native and infective rhizobial population was estimated in average at 80 cells/g soil. In general Senegalese soils in Southern and Eastern zones were less prolific on common bean strains whereas the Niayes zone presented the more important rhizobial population.

Evaluation of potential symbiotic of *Phaseolus vulgaris* bean:

- Selection of elite symbiotic association:

The analysis of variance was conducted at p=0.05 on all data recorded from inoculation, common bean varieties and interaction between these two treatments (Table 2). Significant effects of the three sources of variation were observed on all parameters except nitrogen contents (N%) in shoots. Since interaction between inoculation and varieties was also significant, the main effect of these two factors was not taken in account. Table 3 showed that ARA was more important for the interaction ISRA 350 x Bronco variety with 39 µmol C_2H_4/h/pl. however the high coefficient of variance (>50%; Table 2) did not allow selecting this association. Shoot fresh and dry matters and total N were greater when *Phaseolus vulgaris* bean variety Bronco was inoculated with ISRA 355 rhizobial strain (Table 3). This treatment increased significantly both shoot dry weigh and total N for about 180% in comparison to the average of the non inoculated varieties. The combination of rhizobial strain ISRA 355 x common bean variety Bronco considered as the elite symbiotic association was selected for field experiment set up in the following of this work.

Table 2: F ratios from analysis of variance (ANOVA) of the plant shoots fresh (SFW) and dry (SDW) weights, acetylene reduction activity (ARA) and total nitrogen (total N) of *Phaseolus vulgaris* varieties cultivated in pots and inoculated with *Rhizobium* strain on non sterile Bel Air soil.

Source of variation	dof	SFW	SDW	ARA	N%	total N
Inoculation	10	3.36**	4.12**	3.39**	1.86	113.80**
Varieties	3	5.18*	3.93*	51.40**	1.39	66.43**
Inoculation x Varieties	30	4.17**	3.31**	2.94**	2.12	59.64**
C.V.%		32.9	31.8	50.7	79.1	8.0*

*= significant; ** = highly significant (p =0.05).

- Field inoculation response of common bean *Phaseolus vulgaris*.

In the field in Niayes zone, the *Rhizobium* strain ISRA 355 was associated with the common bean variety Bronco for its high nitrogen fixing potential estimated from the greenhouse experiment. Table 4 showed ANOVA performed on all parameters from treatments. Significant effect of both main factors inoculation and nitrogen fertilizer were observed on all parameters except on PDW and RDW respectively. In addition, the interaction of these treatments was significant on SDW, NDW and ARA. Inoculation of common bean with *Rhizobium* strain ISRA 355 increased the SDW (+27%) and RDW (+22%) in comparison to non inoculated plants (Table 5). A high positive effect of inoculation was recorded when fertilizer was applied at 20 kg N/ha. Pod yield increased for 77% compared to non inoculated and non N fertilized plants (Table 6). NDW was also increased in the same order for 300%. Although, NDW was more important on plants inoculated with strain ISRA 355 than on non inoculated plants. There were non significant difference on pod yield.

When non inoculated plants were compared to inoculated plants in presence of fertilizer applied at 0 and 80 kg N/ha.

Table 3: Plant shoot fresh (SFW) and dry (SDW) weights, acetylene reduction activity (ARA) and total nitrogen (total N) of *Phaseolus vulgaris* varieties cultivated in pots and inoculated with *Rhizobium* strain on non sterile Bel Air soil.

Rhizobium strain	Varieties	SFW (g/pl)	SDW (g/pl)	ARA (mmol/h/pl)	Total N (g/pl)
Control	Bronco	13.6 bcd	0.9 de	14.6 cdefg	0.11 qr
	Rocdor	13.9 bcd	1.1 bcde	15.9 cdefg	0.14 pqr
	Delinel	17.7 bcd	1.1 cde	06.6 efg	0.18 jklm
	Victoire	13.6 bcd	1.2 bcde	05.0 fg	0.16 mno
ISRA 350	Bronco	16.9 bcd	1.6 bcde	39.0 a	0.23 ghi
	Rocdor	13.9 bcd	1.4 bcde	14.9 cdefg	0.24 ghi
	Delinel	14.8 bcd	1.2 bcde	06.9 efg	0.16 mno
	Victoire	15.8 bcd	1.6 bcde	12.4 defg	0.25 fg
ISRA 354	Bronco	10.8 d	2.2 abcde	19.7 cdefg	0.14 opq
	Rocdor	21.0 bcd	0.8 e	16.9 cdefg	0.33 cd
	Delinel	20.9 bcd	1.7 bcde	06.0 fg	0.28 ef
	Victoire	21.0 bcd	2.1 abcde	09.2 efg	0.31 df
ISRA 351	Bronco	11.6 cd	1.7 bcde	21.9 bcdefg	0.13 qr
	Rocdor	15.9 bcd	1.5 bcde	17.6 cdefg	0.22 ghij
	Delinel	21.5 bcd	0.9 de	05.7 fg	0.24 ghi
	Victoire	13.5 bcd	1.5 bcde	10.4 efg	0.24 ghi
ISRA 353	Bronco	20.1 bcd	2.1 abcde	28.8 abcd	0.30 e
	Rocdor	13.6 bcd	1.2 bcde	13.0 defg	0.20 hijk
	Delinel	14.2 bcd	1.3 bcde	09.0 6efg	0.20 hijk
	Victoire	24.1 bcd	2.4 abcd	07.5 efg	0.33 cd
ISRA 355	Bronco	45.0 a	3.1 a	20.2 cdefg	0.42 a
	Rocdor	10.1 d	1.0 de	12.5 defg	0.14 op
	Delinel	11.8 cd	1.1 bcde	03.5 g	0.17 klm
	Victoire	16.3 bcd	1.4 bcde	10.8 efg	0.20 hijk
ISRA 356	Bronco	20.7 bcd	1.8 abcde	12.2 defg	0.26 fg
	Rocdor	17.5 bcd	1.8 abcde	19.8 cdefg	0.29 e
	Delinel	10.8 d	1.0 de	05.6 fg	0.15 nop
	Victoire	15.6 bcd	1.3 bcde	06.0 fg	0.22 ghi
ISRA 357	Bronco	28.7 bc	2.6 ab	13.0 defg	0.38 b
	Rocdor	15.8 bcd	1.7 bcde	30.2 abc	0.23 gh
	Delinel	11.7 cd	1.2 bcde	03.3 g	0.19 ijkl
	Victoire	14.1bcd	1.4 bcde	07.1 efg	0.20 hijk
ISRA 361	Bronco	16.1 bcd	1.5 bcde	21.9 bcdf	0.21 ghi

Table 3: Continued.

	Rocdor	20.1 bcd	2.2 abcde	35.2 ab	0.24 fgh
	Delinel	21.8 bcd	1.6 bcde	10.0 efg	0.25 efg
	Victoire	19.1 bcd	2.2 abcde	09.9 efg	0.38 b
ISRA 362	Bronco	29.9 b	2.6 abc	09.0 efg	0.36 bc
	Rocdor	17.2 bcd	1.7 bcde	24.8 bcde	0.26 fg
	Delinel	23.4 bcd	1.6 bcde	04.3 g	0.25 fg
	Victoire	19.0 bcd	1.9 abcde	05.5 fg	0.31 de
ISRA 363	Bronco	12.3 cd	0.9 de	11.4 efg	0.11 qr
	Rocdor	13.2 bcd	1.3 bcde	18.7 cdefg	0.16 Imn
	Delinel	14.8 bcd	1.2 bcde	05.1 fg	0.14 mnop
	Victoire	09.8 d	1.0 de	06.0 fg	0.14 mnop

For each column, values followed by the same letter do not differ significantly at $p = 0.05$.

- Estimation of nitrogen fixed

The ANOVA performed for N_2 fixed on ARA and Ur-N% showed significant effects of inoculation and N fertilizer at $p = 0.05$ (Table 4). In addition, significant effect of interaction between the treatments was observed on ARA. The amount of N_2 fixed evaluated with Ur-N method was highest on inoculated plants (72.2 g/pl.) than on uninoculated (58.3 g/pl.). Likewise an application of 20 kg N/ha of N fertilizer showed a more important Ur-N% (70.9%) than 0 and 80 kg N/ha fertilizer with 64.2% and 60.6% Ur-N respectively.

The interaction of treatments inoculation and N fertilizer increased ARA (+156%) in plants inoculated with strain ISRA 355 compared to N fertilized plants whatever the rate of N application. This interaction showed a decrease of ARA on plant fertilized with 80 kg N/ha when 20 kg N/ha applied showed the highest value (1.9 µmol/h/pl.) which can be considered as a starting effect on ARA.

Table 4: F ratios from analysis of variance (ANOVA) of the plant shoots (SDW), pods (PDW) roots (RDW) and nodules (NDW) dry weights and acetylene reduction activity (ARA) and the relative abundance of ureide N (Ur-N) of *Phaseolus vulgaris* cultivated in field and inoculated with *Rhizobium* strain and N fertilized.

Source of variation	dof	SDW	PDW	RDW	NDW	ARA/pl.	Ur-N%
Inoculation	1	30.01**	2.0	5.74*	388.15**	916.07**	2719**
Fertilizer N	2	11.22**	5.65*	0.23	75.57**	216.57**	5.12*
Inoc. x Fertilizer	2	2.01	4.65*	0.57	29.63**	153.67**	3.21
C.V.(%)		10.7	18.6	22.3	15.0	7.2	10

* = significant ; ** = highly significant ($p = 0.05$)

Discussion:

Common bean nodulation in Senegal: Some works reported that *Phaseolus vulgaris* bean nodulate erratically world-wide[15]. Investigation on common bean cultivation in Senegal indicated a few indeed, a lack of nodulation in these soils[38]. Plant nodulation in field was observed in three localities where rhizobial inoculation had never been done. Farmers who managed to cultivate the crop did not know about inoculums or theirs benefits. Plants with efficient root nodules from which rhizobial strains were isolated have been cultivated in an experimental trial conducted at Camberène in the Niayes zone by the Professional Formation Center for Horticulture in collaboration with *Tropica Sem*. Nevertheless we have isolated 58 *Rhizobium* strains mainly by trapping with bean plants from soils sampled from different agro-ecological zones in Senegal. Moreover we noted that 42% of soil sampled contained viable and infective *Rhizobium* on common bean when the majority of soils were naturally devoid of bean nodulating *Rhizobium* strains.

Native rhizobial population evaluated by MPN method in the different regions investigated showed a few number of infective bean *Rhizobium* in Senegalese soils. This may be explained by the lack of traditional cultivation of bean in the rural zone inside the country or by the large use of N fertilizer as reported by Giller[14] in the Niayes zone where common bean was cultivated since some decades. However, common bean was considered as a non promiscuous nodulation legume. On the other hand, many leguminous plants such as groundnut (*Arachis hypogaea*) and *Acacia* were largely nodulated by *Rhizobium* strains in these soils[7,5]. Presence of *Rhizobium* that nodulate common bean did not mean the existence of cultural practice of bean plants in these areas. Madrzack *et al.*[22] reported that geographical distribution of *Bradyrhizobium* strains seems to match with the host leguminous. Originated from the Americas, bean *Phaseolus vulgaris* was introduced from Mexico to Europe through Spain in the sixteenth century with some symbiotic *Rhizobium* strains[21,12]. According to Evans[10], *Phaseolus vulgaris* should be introduced in Africa from Brazil during the slavery trade. In Kenya, Portuguese might probably introduce the microsymbionts with seeds of the host plants. However, according to Martinez-Romero and Caballero-Mellado[24], it still seems reasonable to suppose that there has been a natural and successful distribution of rhizobia independent of human action. In Senegal, we are for the moment unaware of the bean introduction history that can contribute to dissemination of its symbionts in the country. However it is some times difficult to determine whether the *Rhizobium* population is native or introduced to an area.

The infectivity of *Rhizobium* strain isolated from *Phaseolus vulgaris* on some endemic leguminous plants in the investigated localities and on many other legumes[17] may explained the existence of symbiotic bacteria in Senegalese soils previously to bean introduction. Clustering of strains from various localities in the same species had been yet reported for *Rhizobium* strains such as those of the cluster U described by de Lajudie *et al.*[5] nodulating *Acacia*, *Leuceana* and *Chamaecrista* and originated in Senegal, Sudan and Brazil.

Table 5: Plant shoots (SDW) and roots (RDW) dry weights of *Phaseolus vulgaris* variety Bronco cultivated in field and inoculated with *Rhizobium* strain ISRA 355 and N fertilized.

Treatments	SDW (g/pl.)	RDW (g/pl.)
Control	5.14b	0.36b
Rhizobium ISRA 355	6.53a	0.44a

For each column, values with the same letter do not differ significantly at $p = 0.05$.

Table 6: Plant pods (PDW) and nodules (NDW) dry weights of *Phaseolus vulgaris* cultivated in field and inoculated with *Rhizobium* strain and N fertilized.

Inoculation	PDW(g/pl.)	NDW(g/pl.)
Control		
0 kg N/ha	4.55b	0.03d
20 kg N/ha	5.92ab	0.05c
80 kg N/ha	5.64ab	0.02d
Rhizobium ISRA 355		
0 kg N/ha	5.64ab	0.10b
20 kg N/ha	8.06a	0.20a
80 kg N/ha	5.65ab	0.09b

For each interaction, values with the same letter are not significantly different at $p = 0.05$.

Inoculation of common bean *Phaseolus vulgaris* in Senegalese soils: Since MPN method showed that Senegalese soils were naturally low on native and infective common bean *Rhizobium*, inoculation of the crop may be envisioned. Thereby significant results obtained from ANOVA on the response of common bean varieties inoculated with strains of *Rhizobium* allowed the selection of elite symbiotic association. This approach of selection was proposed for enhancing the response of legume plants to inoculation with strains of rhizobia[37,9]. Greenhouse experiment in our conditions showed variable response of the four bean cultivars when inoculated with ten rhizobial strains. This result can be compared to those obtained in other works on different common bean varieties[19,15]. The association *Rhizobium* strain ISRA 355 and the common bean variety Bronco was selected for field trials. Therefore reproducibility of results obtained from greenhouse to field was often discussed. Beatie *et al.*[2] reported highly significant correlation on results obtained on bean in the laboratory on sterile and non sterile supports and in field in three sites. Zaman-Allah *et al.*[40] reported that field trials substantiated the glasshouse observations and emphasized the effects of cultivar-rhizobia interactions on symbiotic parameters and yields. Since this elite association was selected on 40 possible combinations according to high symbiotic performance, it was maintained for field experiment. In our experimental conditions, SDW, NDW and pod yields were highest in plants inoculated and amended with 20 kg N/ha compared to uninoculated plants. Pineda and Kipe Nolt[28] have been yet obtained an increase of common bean yield by selection of rhizobial strains. Different results were reported on two soybean *Glycine max* varieties inoculated with *Bradyrhizobium* strains in greenhouse experiment[29]. However the effect of plants genotype on biological nitrogen fixation was reported. In this way, Graham[15]. and Redden *et al.*[30] have indicated that diver genotypes of legume varieties such as common bean were different in nodulation capacity and N_2 fixation. Our finding showed an increase of PDW (21%) and NDW (29%) on plants inoculated with ISRA 355 compared to uninoculated plants. A positive effect of inoculation was so obtained on bean *Phaseolus vulgaris* in Senegal such as in Cameroon[32] and in Kenya[34]. In Tanzanian soils, grain yields of common bean were markedly increased from inoculation compared to uninoculated unfertilised plots[26]. However, results reported by Abebe and Abegaz[1] showed that nitrogen fixed by common bean was not sufficient to enhance pod yield and these authors suggested to apply 40 to 100 kg N/ha. In semi-arid soils in Southern-east of Kenya, the inoculated common bean and N application treatment recorded the largest seed dry weights[23]. In our study, a significant interaction between ISRA 355 and Bronco increased pod yields for 43% in presence of 20 kg N/ha fertilizer. Ssali and Keya[35] showed that 10 kg N/ha increased the amount of N fixed and pod yield on common bean. Streeter[36] reported that a few level of fertilizer N stimulate bean growth and enhance nodule mass. For this author this N application level was necessary to maximize crop yields. In Brazil, Hungria *et al.*[20] observed a synergistic effect between low levels of N fertilizer and inoculation with superior strains, resulting in yield increases in two experiments. These authors showed that nodulation and N_2 fixation rates evaluated by total N and N-ureide increased when common bean plants were inoculated with selected strains in relation to the indigenous rhizobial population. At Bel Air station where soil nitrogen is low (0.026%), common bean cultivated with application of 20 kg N/ha increased nitrogen fixed evaluated with Ur-N (+10%) and ARA (+101%). These results confirm those obtained on common bean by Diouf *et al.*[8] in Senegal using N dilution isotope techniques. It is known that, high N level in soil inhibit inoculation effect[35]. Diatloff *et al.*[6] reported that 100 kg N/ha fertilizer decreased the rate of ureides in common bean xylem sap. In our study, N fertilizer supplied at 80 kg N/ha lower than 100 kg N/ha suggested by Diatloff *et al.*[6] and Ssali and Keya[35] decreased effect of inoculation.

Senegalese soils contained small population of *Rhizobium* strains infective on common bean *Phaseolus vulgaris*. The elite symbiotic association *Rhizobium* strain ISRA 355 and bean variety Bronco pointed out that the optimization of the effect of inoculation in Niayes zone lies in identifying and matching bean varieties to rhizobial strains. In addition, N fertilizer applied at 20 kg N/ha increased plant growth, N_2 fixed and pod yields when 80kg N/ha of N fertilizer decreased nodulation and affected negatively pod yields.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Mr. Omar Touré and Mrs. Marie-Claire Da Sylva for their valuable and useful technical assistance.

REFERENCES

1. Abebe, A. and B. Abegaz, 1985. Nitrogen fixation of two *Phaseolus vulgaris* varieties at different rates of inoculation. In Biological Nitrogen Fixation in Africa. Proceedings of the first Conference of the AABNF. Ssali, H. and S.O. Keya eds. MIRCEN, Nairobi, 313-323.
2. Beattie, G.A., Clayton, M.K. and J. Handelsman, 1989. Quantitative comparison of laboratory and field competitiveness of *Rhizobium leguminosarum* biovar phaseoli. Appl. Environ. Microbiol., 55: 2755-2761.
3. Boddey, R.M., Pereira, J.A.R., Hungria, M. Thomas, R.J. and M.C.P. Neves, 1987. Methods of the nitrogen assimilation and transport in grain legume. MIRCEN J., 3: 3-22.
4. Brockwell, J., 1982. Plant-infection counts of rhizobia in soil. In Nitrogen Fixation in Legumes. J.M. Vincent, ed. Academic Press, New York, 41-58.
5. de Lajudie, P., A. Willems, B. Pot, D. Dewettinck, G. Maestrojuan, M. Neyra, M.D. Collins, B. Dreyfus, K. Kersters and M. Gillis, 1994. Polyphasic taxonomy of rhizobia: Emendation of the genus *Sinorhizobium* and description of *Sinorhizobium saheli* sp. nov. and *Sinorhizobium teranga* sp. nov. Int. J. Syst. Bacteriol., 44: 715-733.
6. Diatloff, A., Redden, R.J. and D.F. Herridge, 1991. Correlation between xylem ureide levels and nodulation in field-grown *Phaseolus vulgaris*. Aust. J. Exp. Agr., 31: 679-682.
7. Diouf, A., 1993. Evaluation de la performance symbiotique de souches de *Bradyrhizobium* pour l'inoculation de l'arachide (*Arachis hypogaea* L.) au Sénégal. Mémoire de Diplôme d'Etudes Approfondies, UCAD, Dakar, 42.
8. Diouf, A., I. Ndoye, M.M. Spencer, C. Nef-Campa, and M. Gueye, 1999. Need for inoculation of common bean (*Phaseolus vulgaris* L.) in Senegal and assessment of nitrogen fixation using 15N isotope dilution technique. Symbiosis, 27: 251-257.

9. Dommergues, Y., L.M. Bordeleau, et M. Gueye, 1990. Les recherches sur la fixation biologique de l'azote et leur impact présent et futur sur la production végétale et forestière en Afrique. In Maximiser la fixation biologique de l'azote pour la production agricole et forestière en Afrique. M. Gueye, K. Mulogoy, et Y. Dommergues eds. IIIème Conférence de l'AABNF, Dakar, Sénégal, 352-355.
10. Evans, A.M., 1976. Beans *Phaseolus* spp. (*Leguminosae-Papilionasae*). In Evolution of Crop Plants. Simmonds N.W. ed. Longman, London, pp. 168-172.
11. Gepts, P., 1990. Biochemical evidence bearing on the domestication of *Phaseolus* (*Fabaceae*) bean, Econ. Bot., 44: 28-38.
12. Gepts, P. and F.A. Bliss, 1988. Dissemination pathways of common bean (*Phaseolus vulgaris*, *Fabaceae*) deduced from phaseolin electrophoretic variability. II. Europe and Africa. Econ. Bot., 42: 86-104.
13. Gibson, A.H., 1963. Physical environment and symbiotic nitrogen fixation. I. The effect of temperature on recently nodulated *Trifolium subterraneum* L. plants. Aust. J. Biol. Sci., 16: 1179-1188.
14. Giller, K.E., 1990. Assessment and improvement of nitrogen fixation in tropical *Phaseolus vulgaris* L. Soil Use and Management, 6: 82-84
15. Graham, P.H., 1981. Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L. Field Crops Res., 4: 93-112.
16. Hardy, W.F., R.C. Burns, and R.D. Holsten, 1973. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem., 5: 47-81.
17. Hernandez-Lucas, I., L. Segovia, E. Martinez-Romero, and S.G. Pueppke, 1995. Phylogenetic relationships and host range of *Rhizobium* spp. that nodulate *Phaseolus vulgaris* L. Appl. Environ. Microbiol., 61: 2775-2779.
18. Herridge, D.F. and M.B. Peoples, 1990. Ureide assay for measuring nitrogen fixation by nodulated soybean calibreted by 15N methods. Plant Physiol., 93: 495-503.
19. Holl, F.B., 1975. Host plant control of the inheritance of dinitrogen fixation in the *Pisum-Rhizobium* symbiosis. Euphytica., 24: 767-770.
20. Hungria, M., Campo, R.J. and I.C. Mendes, 2004. Benefits of inoculation of the common bean (*Phaseolus vulgaris*) crop with efficient and competitive *Rhizobium tropici* strains. Biol. and Fert. of Soils., 39: 88-93.
21. Kaplan, L., 1981. What is the origin of the common bean? Econ. Bot., 19: 358-368.
22. Madrzak, C.J., B. Golinska, J. Kroliczak, K. Pudelco, D. Lazewska, B. Lampka, and M.J. Sadowsky, 1995. Diversity among field populations of *Bradyrhizobium japonicum* in Podland. Appl. Environ. Microbiol., 61: 1194-1200.
23. Maingi, J.M., C.A. Shisanya, N.M. Gitonga, and B. Hornetz, 2001. Nitrogen fixation by common bean (*Phaseolus vulgaris* L.) in pure and mixed stands in semi-arid south-east Kenya. European Journal of Agronomy, 14: 1-12.
24. Martinez-Romero, E. and J. Caballero-Mellado, 1996. *Rhizobium* phylogenies and bacterial genetic diversity. Crit. Rev. Plant Sci., 15: 113-140.
25. Mitchell, H.L., 1972. Micro determination of nitrogen in plant tissues. J. Assoc. Off. Agr. Chem., 55: 1-3.
26. Ndakidemi, P.A, F.D. Dakora, E.M. Nkonya, D. Ringo, and H. Mansoor, 2006. Yield and economic benefits of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) inoculation in northern Tanzania. Australian Journal of Experimental Agriculture, 46: 571-577.
27. Neil, W.S., 1936. Adaptation of the micro-Kjeldahl method for the determination of nitrogen in plant tissues. Plant Physiol., 11: 173-179.
28. Pineda, P. and J. Kipe Nolt, 1990. Response of bean varieties to inoculation with selected *Rhizobium* strains in El salvador. Turrialda., 40: 410-415.
29. Pinochet, X., F. Arnaud and J.C. Cleyet-Marel, 1993. Competition for nodule occupancy of introduced *Bradyrhizobium japonicum* strain SMGS1 in French soils already containing *Bradyrhizobium japonicum* strain G49. Can. J. Microbiol., 39: 1022-1028.
30. Redden, R.J., A. Diatloff, and T. Usher, 1990. Field screening accessions for *Phaseolus vulgaris* for capacity to nodulate over a range of environments. Aust. J. Exper. Agr., 30: 265-270.
31. Rosen, H., 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys., 67: 10-15.
32. Salez, P. and H. Saint Macary, 1987. Inoculation du haricot (*Phaseolus vulgaris*) par *Rhizobium phaseoli* au Cameroun. Agr. Trop., 42: 269-274.
33. Snell, F.D. and Snell C.T., 1949. Colorimetric methods of analysis. (2) Inorganic determination. Van Nostrand Reinhold ed., New York, 950.
34. Ssali, H., 1988. *Rhizobium phaseoli* inoculation trials on farmer's fields in Kenya. East Afr. Agr. For. J., 53: 151-157.
35. Ssali, H. and S.O. Keya, 1986. The effects of phosphorus and nitrogen fertilizer level on nodulation, growth and dinitrogen fixation of three bean cultivars. Trop. Agric., 63: 105-109.

36. Streeter, J.G., 1988. Inhibition of legume nodule formation and N2 fixation by nitrate. Crit. Rev. Plant Sc., 7: 1-23.
37. Trinick, M.J., 1982. Host-*Rhizobium* associations. In. Nitrogen fixation in legumes. Vincent, J.M. ed. Academic Press Australia.
38. Wey, J., 1981. La fixation biologique de l'azote par la symbiose *Rhizobium*-légumineuse. Synthèse des résultats de la campagne 1980. Prog. Rech., 2-4-13. ISRA. 8.
39. Young, E.C., and C.F. Conway, 1942. On the estimation of allantoin by the Rimimi-Schryver reaction. J. Biol. Chem., 142: 839-853.
40. Zaman-Allah, M., B. Sifi, B.L Taief, M.H. El Aouni, and J.J. Drevon, 2007. Rhizobial inoculation and P fertilization response in common bean (*Phaseolus vulgaris*) under glasshouse and field conditions. Exper. Agric., 43: 67-77.