Vol. 11(4), pp. 153-163, October-December 2020
DOI: 10.5897/JSSEM2020.0833
Article Number: 247787065540
ISSN: 2141-2391

http://www.academicjournals.org/JSSEM

ACADEMIC JOURNALS
expand your knowledge

Journal of Soil Science and Environmental Management

Full Length Research Paper

Effect of rhizobial inoculation combined with phosphorus fertilizer on nitrogen accumulation, growth and yield of soybean in Benin

Houngnandan H. B.[1,2*], Adandonon A.[2], Akplo T. M.[1], Zoundji C.C.[1], Kouelo A. F., Zeze A.[3], Houngnandan P.[1,2], Bodjrenou R.[1, 2], Dehoue H.[1, 2] and Akinocho J.[1,2]

[1]Laboratoire de Microbiologie des Sols et d'Ecologie Microbienne, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi (LMSEM/FSA/UAC); BP 711 Abomey-Calavi, République du Bénin.
[2]Ecole de Gestion et de Production Végétale et Semencière, Université Nationale d'Agriculture (EGPVS/UNA); BP 43 Kétou, République du Bénin.
[3]Laboratoire de Biotechnologies Végétale et Microbienne, Unité Mixte de Recherche et d'Innovation en Sciences Agronomiques et Génie Rural, Institut National Polytechnique Felix Houphouët-Boigny (INPHB, Côte d'Ivoire), BP 1093 Yamoussoukro Côte d'Ivoire.

Received 5 July 2020; Accepted 6 November 2020

The objective of this study was to determine the amount of phosphorus application needed for the Bradyrhizobium inoculation to improve soybean N uptake and grain yield. Two factors were investigated: inoculation with *Bradyrhizobium diazoefficiens* strain USDA 110 and application of phosphorus fertilizer with different doses (0; 50; 100 and 150 kg ha^{-1} P_2O_5). Trials were carried out in two agro-ecological zones (ZAE) in Benin with two experimental sites namely Kétou and Zè. The experimental design was a Complete Randomized Block Design. Results showed that the highest mean height values were recorded T1+150P (42.22 cm at Zè and 43.82 cm at Kétou) while the lowest values were recorded with the treatment T0 + 0P. Treatment T0 +100P gave the highest aboveground biomass at 10 weeks after sowing at Kétou (2521.43 kg DM ha^{-1}) while T1+150P recorded the highest aboveground biomass at Zè (3392.75 kg DM ha^{-1}). At Kétou, the grain yield was increased under T1+100P by 98% compared with T0+0P. At Zè, the treatments T1+100P significantly increased the soybean grain yield by 147% compared with T0+0P. Likewise, T1+100P allowed to have the highest total nitrogen yields in the grain which is respectively 144.13 kg ha^{-1} N and 295.02 ha^{-1} kg N at Kétou and Zè. Overall, the inoculation with *B. diazoefficiens* had significantly increased the number of nodules for all of the phosphorus fertilizer amounts. The mycorhization frequency was significantly lower under the treatment T0 + 0P (41.46%) than the other treatments while that was higher under the treatment T1+50P (76.04%).

Keywords: *Bradyrhizobium diazoefficiens*, soybean inoculation, grain yield, Benin.

INTRODUCTION

Soybean (*Glycine max* (L) Merill) is a food legume with many agronomic and nutritional benefits (Ogumniyi et al.,

*Corresponding author. E-mail: bhoungnandan@yahoo.fr.

2012). Sometimes, it is considered as a miracle plant (Aganze, 2014), and one of the world's fastest-growing major crops, contributing to human nutrition in terms of energy and the most important source of protein for human and animal nutrition (Zoundji et al., 2015). In Benin, soybean is considered as an emerging crop in the Government Action Program (PAG, 2016) and is included in most of the Benin's Agricultural Development Poles. Soybean production is increasing and reached 72891 tons in 2015 (Kpenavoun et al., 2018). However, the current levels of soybean yields are still very low (1100 kg ha^{-1}) (IAEA, 2020) as compared to the potential yield of 4000 kg ha^{-1}, and consequently do not meet the growing need (Kpenavoun et al., 2018). This low yield level observed in Benin is mainly attributed to some factors such as decrease in soil fertility with deficiency of nitrogen and phosphorus, low agricultural practices and erosion or adverse weather conditions (Index Box., 2019).

Growing bodies of research over the last past years have shown that, despite the low soil fertility, exported nutrients are not adequately replaced in most cases (Akedrin et al., 2020). This situation leads to a drastic deficiency in nitrogen, phosphorus and potassium and results in low yields (Javaheri and Baudoin, 2001; Houngnandan et al., 2001). As with all other legumes, soybean has the potential to fix nitrogen in symbiosis with *Rhizobium* (Vieira et al., 2010). Under optimal conditions, soybean can set up to about 450 kg N ha^{-1}, limiting the need for synthetic nitrogen fertilizer (Giller, 2001). Thus, inoculation of efficient strains of nitrogen-fixing symbiotic bacteria to the soybean seeds before sowing would be crucial for the improvement of the crop productivity.

Phosphorus deficiency in soil constitutes limiting factor for plant growth (Seyni and Laouali, 2020). The development of nodules, the growth of the plant and its ability to fix atmospheric nitrogen are reported to be largely influenced by the assimilable phosphorus content of the soil (Giller and Dashiell, 2007; Abbassi et al., 2010). It is also said that the process consumes large amounts of energy (Schulze et al., 2006) and energy-generating metabolism strongly depends on the availability of P (Plaxton, 2004; Abassi et al., 2010). Moreover, Waluyoa et al. (2004) reported that P is essential for nodulation as it increases the number of nodule primordia. Organogenesis of nodules concentrates large quantities of phosphorus in phospholipid membranes and bacterial and plant nucleic acids (Drevon, 2017). However, Graham and Rosas (1979) and Jacobsen (1985) reported that phosphorus fertilization increases symbiotic nitrogen fixation by stimulating plant growth rather than exerting a direct influence on initiation, growth, development and function of nodules. Although the plant responds well to P application, the effective doses vary according to authors. Moreover, there are several studies showing interaction between Rhizobia inoculation and phosphorus intake. Thus, Ndakidemi et al. (2006), Abbasi et al. (2010) and Akpalu et al. (2014) reported that combination of beneficial soil bacteria and phosphorus in legumes significantly increased nodulation, pod formation, grain development and yield compared to a single intake of phosphorus. It was also shown that inoculation with *Rhizobium* bacteria and phosphorus supplementation improved the uptake of macronutrients (N, P, K) into different organs of the whole soybean plant (Abbasi et al., 2010; Zoundji et al., 2015; Tairo and Ndakidemi 2014). Highly efficient and competitive *Rhizobium* strains and adequate phosphorus intake (Scherer et al., 2008) could then significantly increase legume growth and nitrogen fixation. This effective dose of P to supply is yet to be determined. Zoundji et al. (2015) reported that inoculation with Bradyrhizobium strains and 50 kg kg ha-1 P2O5 increased soybean growth and yield. But, increasing P dose supply beyond 50 kg reported by Zoundji et al., 2015 may lead to more increase of soybean yield. So far, to the best of the authors' knowledge, there is no information about the optimum dose required to get maximum yield of the crop. Thus, research study is needed to determine the optimum dose of P for maximum yield of soybean. The objective of the current study was to assess the effect on soybean growth, yield and nitrogen accumulation of the application of different phosphorous doses in combination with inoculation of *B. diazoefficiens* in two agro-ecological zones in Benin.

MATERIALS AND METHODS

Study area

The study was carried out at Kétou (7° 41'17 "N and 2° 47'40 "E) in the 5th agroecological zone and Zè (6° 87'00" N and 2° 26'00"E) in the 6th agroecological zone of Benin (Figure 1). Areas were chosen on the basis of a random draw taking into account zones with low soybean production. The rainfall pattern is bimodal in the two areas: long rain season (LR) lasting from March to June and short rain season (SR) from September to November. Cumulatively, the site of Ze received 427.23 mm whilst 583.9 mm was recorded in rainfall at Kétou during the experiment (Figure 2). The major characteristics of the soil are presented in Table 1. The predominant soil type in both sites is Ferralsols which are well drained and deep. At Kétou, the soil is sandy-clay-loamy while it is sand-loamy with low fertility. The primary land use activities are subsistence farming, livestock rearing, cash crop farming, and agroforestry. The primary farming system in the area is mixed farming.

Experimental design

The experimental design was a Complete Randomized Block Design (RCBD) with two factors. The first was inoculation with two levels: inoculated and non-inoculated. The second was phosphorus application with four levels namely 0, 50, 100 and 150 kg ha^{-1} of P_2O_5. The treatments were four-replicated within each site. The experiment was carried-out from July to October 2018. The plot sizes were 6 m by 4 m. Land preparation was done using a tractor to 25 cm depth. Planting was done at 0.50 m by 0.20 m in both sites. Soybean (*Glycine max* L.) variety TGX 1910-14F was used as test crop. Three seeds were planted per hill to ensure maximum plant population. Uniform dose (20 kg N/ha) of Urea fertilizer was

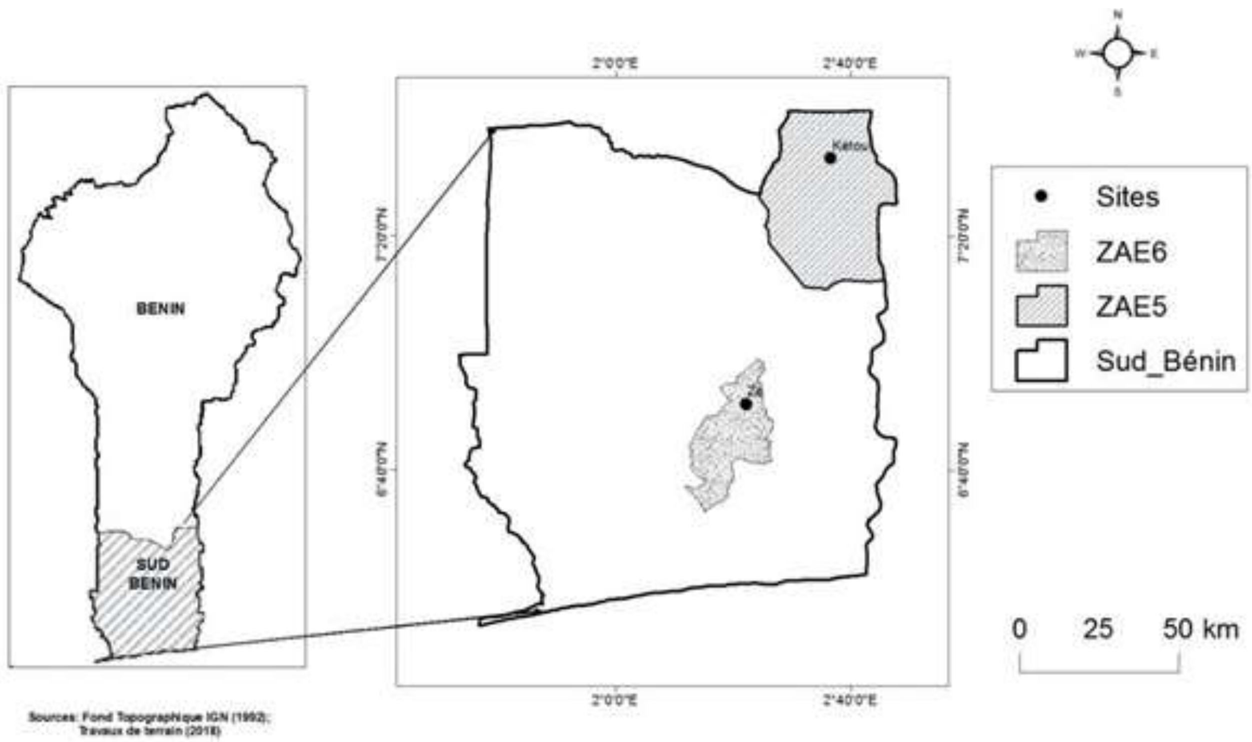

Figure 1. Distribution map of the experimental sites.

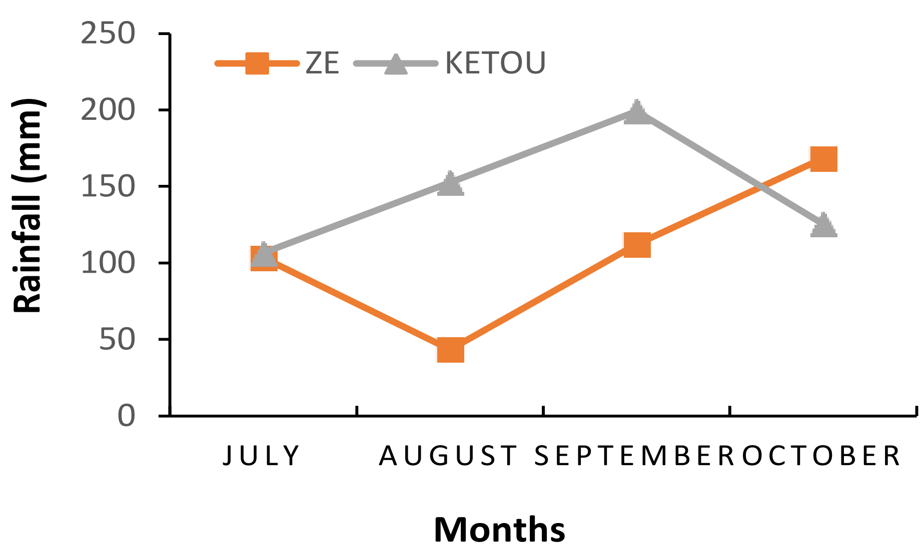

Figure 2. Rainfall of study areas.
Data Source: METEO-BENIN (2018).

Table 1. Initial Physical and chemical properties of the soil.

AEZ	Locality	pH (H_2O)	CO (%)	N (%)	Available P (ppm)	Silt (%)	Clay (%)	Sand (%)
AEZ 5	Ketou	6.20	0.54	0.05	31.11	23.10	25.02	51.88
AEZ 6	Zè	6.08	0.97	0.16	10.55	4.30	20.01	75.70

AEZ: Agro-ecological zone; pH: Soil pH water; CO: Organic carbon; N: Nitrogen; P: Phosphorus.

applied as nitrogen source. The fields were hand-weeded four weeks after planting (WAP) and thereafter when needed.

Seed inoculation with the strain *Bradyrhizobium diazoefficiens* USDA 110 was done before planting at the rate of 100 g peat per 15 kg of soybean seed. The strains were obtained from the IITA rhizobium collection. They were grown in YEMA solution (Yeast Extract-Mannitol-Agar Medium, Vincent, 1970) and then incubated for 5 days. The resulting bacterial colonies were transferred to an Erlenmeyer flask containing YEM liquid culture solution (Yeast Extract-Mannitol, Vincent, 1970) and placed on a magnetic stirrer for 7 days. Furthermore, 80 grams of peat were mixed with the culture medium obtained after the 7 days with a density of 108 viable cells per milliliter. The phosphorus was applied in the form of Triple Super Phosphate TSP at 46% P_2O_5 and at four levels: 0kg ha^{-1} P_2O_5; 50 kg ha^{-1} P_2O_5; 100 kg ha^{-1} P_2O_5 and 150 kg ha^{-1} P_2O_5. The application was done 11 days after soybean was planted. A line was drawn 5cm from the holes and parallel to the sowing line. The fertilizer doses previously weighed in bags for each plot were applied continuously in these lines, avoiding the appearance of manure on the surface.

Data collection

As for data collection, four pockets were randomly selected on four different lines. Plant height at 15, 30 and 45 days after sowing (DAS) were recorded. For the determination of biomass yield at the flowering stage, net area of $1m^2$ were made on each plot at 10 Weeks After Sowing (10 WAS). The plants in the net area were harvested and weighed to determine the total weight of the biomass. The samples were oven-dried at 65 ° C for 72 hours and weighed after drying. The yield of biomass with respect to the dry matter (DM) was determined according to the equation proposed Kouelo et al. (2012):

$$Rb = [(Po \times Pt) / (P1 \times NA)] \times 10,000$$

With Rb: biomass yield at flowering; Po: Dry weight of the biomass sample; P1: Fresh weight of the biomass sample; Pt = Total wet weight of biomass on the usable area and NA: Net Area. The soybean plants were harvested at maturity over an area of 10.5 m^2 (3.5 m × 3 m). On each experimental plot, a distance of 1.5 m was left on each side of the length and a distance of 0.25 m on each side of the width to avoid edge effects. Grain samples were taken and oven dried at 65°C for 72 h. Grain yield was determined according to the following equation (Kouelo et al., 2012):

$$R\ (kg\ DM / ha) = PF\ (kg) \times [10,000\ (m^2 / ha) / N\ area\ (m^2)] \times [PS\ (kg) / PH\ (kg)]$$

With R: the yield of the dry matter (grain); PF: the fresh weight of the grains harvested on the useful surface or harvest area; PS: the sample dry weight; and PH: the fresh weight of the sample grain. Nitrogen content in aboveground biomass and grain was determined by the Kjeldahl method. For the determination of microbiological parameters, roots were harvested 10 weeks after sowing in plot units and used to measure mycorrhization frequencies (F%) and intensities (I%) according to the method of Phillips and Hayman (1970) modified by Trouvelot et al. (1986).

Statistical analysis

Collected data were subjected to multivariate analysis of variance (MANOVA) using the agricolae and lawstat packages in R software (version 3.4.0). For treatments means comparison, Student Newman Keuls's post hoc test was used at p= 0.05.

RESULTS

Effect of *B. diazoefficiens* inoculation and mineral phosphorus fertilization on agronomic parameters of soybean

The combination of inoculation and phosphorus fertilization significantly ($p < 0.05$) affected soybean height at 45 Days After Sowing (DAS). At both experimental sites, the highest mean values of the soybean height were recorded with the treatment T1+150P (42.22 cm at Zè and 43,82 cm at Kétou) while the lowest values were recorded with the treatment T0+0P (23.63 cm at Zè and 32.75 cm at Kétou). At Kétou, the height of soybean plant was increased under T0+50P; T0+100P; T0+150P; T1+0P; T1+50P; T1+100P and T1+150 by 11; 16; 37; 20; 21; 46 and 85% compared with T0+0P. However, the difference between the height obtained under the treatments T0+50P; T0+100P; T1+0P and T0+50P was not significant (Table 2). Likewise, the heights observed under T0+150 and T1+100P were not significantly difference. At Zè, the height of soybean plant was increased under T0+50P; T0+100P; T0+150P; T1+0P; T1+50P; T1+100P and T1+150 by 19; 26; 21; 11; 19; 23 and 29% compared with T0+0P. The treatments T0+50P; T0+150P; T1+100P are statistically equal.

Inoculation with *B. diazoefficiens* and phosphorus fertilization interacted to significantly affect the aboveground biomass of the soybean at 10 Weeks after Sowing (WAS) in both experimental sites (Table 3). Treatment T0+100P yielded the highest aboveground biomass of the soybean at 10WAS in the site of Kétou (2521.43 kg DM ha^{-1}) while T1+150P yielded the highest aboveground biomass at Zè (3392.75 kg DM ha^{-1}). Conversely, the lowest aboveground biomass was yielded under the treatment T0+0P in both sites (1182.99 at Kétou and 1737.32 at Zè). As for the root biomass, the interactive effect of Inoculation with *B. diazoefficiens* and phosphorus fertilization was significant only in the site of Zè where the root biomass recorded under T0+0P was lower that recorded under the other treatments (Table 3). The root biomass was significantly increased under T0+50P; T0+100P; T0+150P; T1+0P; T1+50P; T1+100P and T1+150 by 28; 32; 32; 7; 57; 32 and 75% compared with T0+0P.

The combination (Inoculation with *B. diazoefficiens* *phosphorus fertilization) significantly affected the soybean grain yield at Kétou as well as at Zè (Table 5). In both sites, T1+100P yielded the highest grain amount compared with the other treatments. At Kétou, the grain yield was increased under T0+50P; T0+100P; T0+150P; T1+0P; T1+50P; T1+100P and T1+150 by 19; 41; 71; 54; 54; 98 and 61% compared with T0+0P. At Zè, the treatments T0+50P; T0+100P; T0+150P; T1+0P; T1+50P; T1+100P and T1+150 significantly increased the soybean grain yield by 11; 42; 36; 27; 91; 147 and 89% compared with T0+0P. The grain yield recorded under the treatments T1+50P and T1+150 were statistically

Table 2. Effect of *Bradyrhizobium diazoefficiens* strain and phosphorus supply on soybean height.

Treatments [(1)]		**Plants height (cm)**					
		15DAS		**30DAS**		**45DAS**	
Ino	P	AEZ5 Kétou	AEZ6 Zè	AEZ5 Ketou	AEZ6 Ze	AEZ5 Ketou	AEZ6 Ze
T0	0P	9.78 ± 1.24[a]	9.68 ± 1.23[a]	15.28 ± 1.84[a]	24.19 ± 5.74[a]	23.63 ± 3.54[c]	32.75 ± 1.83[a]
	50P	10.20 ± 0.58[a]	9.75 ± 0.41[a]	15.01 ± 1.72[a]	27.75 ± 2.27[a]	26.15 ± 3.15[bc]	39.03 ± 0.74[b]
	100P	10.55 ± 1.18[a]	10.08 ± 1.62[a]	15.43 ± 2.35[a]	28.41 ± 4.43[a]	27.32 ± 3.45[bc]	41.32 ± 0.75[ab]
	150P	10.13 ± 1.44[a]	9.99 ± 0.43[a]	17.26 ± 1.21[a]	28.26 ± 3.46[a]	32.43 ± 2.52[ab]	39.78 ± 1.78[b]
T1	0P	9.90 ± 1.37[a]	10.66 ± 0.60[a]	16.18 ± 2.39[a]	26.80 ± 3.76[a]	28.45 ± 3.08[bc]	36.20 ± 0.51[b]
	50P	10.10 ± 0.95[a]	10.64 ± 0.40[a]	15.70 ± 1.76[a]	26.33 ± 3.02[a]	28.60 ± 2.21[bc]	38.88 ± 0.5d
	100P	10.30 ± 0.76[a]	9.63 ± 0.36[a]	16.30 ± 2.00[a]	27.51 ± 3.80[a]	34.60 ± 2.06[a]	40.14 ± 0.91[b]
	150P	10.40 ± 0.50[a]	10.60 ± 0.44[a]	15.74 ± 1.93[a]	28.33 ± 3.02[a]	43.82 ± 1.36[ab]	42.22 ± 1.27[c]
	F-value	0.23	1.18	0.53	0.56	5.66	21.34
	p-value	0.973	0.349	0.796	0.780	< 0.001	< 0.001

[(1)]Mean ± standard error. T1: With Inoculation of B diazoefficiens; T0: without Inoculation of B diazoefficiens; 0P: 0 kg ha^{-1} P_20_5; 50P: 50 kg ha^{-1} P205; 100P: 100 kg ha^{-1} P_20_5; 150P: 150 kg ha^{-1} P205; AEZ: Agro Ecological Zone; DAS: Day after Sowing. For each factor, same superscript letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the post hoc SNK test performed in case effects if the model were significant (p≤0.05).

Table 3. Effect of Bradyrhizobium diazoefficiens strain and phosphorus supply on biomass yield.

Treatment [(1)]		**Biomass yield at 10 WAS**			
		Aerial (kg DM ha^{-1})		**Root (kg DM ha-1)**	
Ino	P	AEZ5 Ketou	AEZ6 Ze	AEZ5 Ketou	AEZ6 Ze
T0	0P	1182.99±65.52[d]	1782.84±53.21[d]	312.55±23.19[a]	306.95±6.22d
	50P	1569.31±112.18[c]	2283.08±78.21[c]	314.00±134.13[a]	393.64±10.48[c]
	100P	1896.32±144.03[cb]	2708.25±127.70[b]	323.30±138.4[a]	405.91±13.74[c]
	150P	2106.80±494.38[b]	2237.06±33.83[c]	407.05±98.36[a]	405.36±25.52[c]
T1	0P	1620.45±93.29[c]	1737.32±10.1[d]	326.35±63.26[a]	328.98±30.25d
	50P	1845.07±75.55[cb]	2684.04±58.49[b]	332.55±43.55[a]	483.39±17.77[b]
	100P	2521.43±149.21[a]	2279.29±76.65[c]	421.60±98.67[a]	404.52±5.24[c]
	150P	2005.62±182.75[cb]	3392.75±144.45[a]	417.58±18.67[a]	537.33±30.99[a]
	F-value	1.15	164.17	1.21	55.72
	p-value	0.0142	< 0.001	0.332	< 0.001

[(1)]Mean ± standard error. T1: With Inoculation of B diazoefficiens; T0: without Inoculation of B diazoefficiens; 0P: 0 kg ha^{-1} P_20_5; 50P: 50 kg ha^{-1} P_20_5; 100P: 100 kg ha^{-1} P_20_5; 150P: 150 kg ha^{-1} P_20_5; AEZ: Agro Ecological Zone; DM: Dry Weigh. For each factor, same superscript letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the post hoc SNK test performed in case effects if the model were significant (p≤0.05).

Table 4. Effect of *Bradyrhizobium diazoefficiens* strain and phosphorus input on Nitrogen yield.

Treatment [(1)]		N Total yield AT 10WAS (kg N ha^{-1})			
		Aboveground Biomass		Grain	
Ino	P	AEZ5 Kétou	AEZ6 Zè	AEZ5 Kétou	AEZ6 Zè
T0	0P	24.98 ± 3.66^{d}	39.12 ± 0.90^{d}	68.11±9.24^{e}	92.26 ± 4.56^{e}
	50P	25.87 ± 1.92^{d}	50.87 ± 4.19^{c}	77.30±12.29^{de}	108.61 ± 7.58^{de}
	100P	34.67 ± 2.54^{bc}	62.48 ± 4.63^{b}	94.16±10.95^{cd}	133.18 ± 16.72
	150P	37.08 ± 2.00^{bc}	43.81 ± 2.20^{d}	90.15±19.59^{cde}	134.98 ± 15.40
T1	0P	31.48 ± 8.11^{c}	66.03 ± 1.06^{b}	81.06±9.17^{de}	138.38 ± 17.79^{d}
	50P	32.93 ± 2.72^{c}	92.06 ± 6.34^{a}	110.94±12.44^{cb}	183.81 ± 14.78^{c}
	100P	59.19 ± 6.23^{a}	63.73 ± 3.37^{b}	144.13±11.26^{a}	295.02 ± 13.29^{a}
	150P	40.58 ± 31^{b}	96.96 ± 2.01^{a}	123.20±9.40^{b}	211.49 ± 28.83^{b}
F-value		40.19	138.77	13.43	65.11
p-value		<0.001	<0.001	0.0130	< 0.001

[(1)]Mean ± standard error. T1: With Inoculation of B. diazoefficiens; T0: without Inoculation of B diazoefficiens; 0P: 0 kg ha^{-1} P_20_5; 50P: 50 kg ha^{-1} P_20_5; 100P: 100 kg ha^{-1} P_20_5; 150P: 150 kg ha^{-1} P_20_5; AEZ: Agro Ecological Zone. For each factor, same superscript letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the post hoc SNK test performed in case effects if the model were significant (p≤0.05).

Table 5. Effect of *Bradyrhizobium diazoefficiens* strain and phosphorus input on grain yield.

Treatment [(1)]		Grain yield (kg DM ha^{-1})	
Ino	P	AEZ5 Kétou	AEZ6 Zè
T0	0P	1101.40 ± 114.75^{e}	1668.58 ± 33.35^{e}
	50P	1644.34 ± 220.67^{c}	1848.59 ± 113.85^{e}
	100P	1275.81 ± 149.32^{de}	2375.74 ± 149.34^{c}
	150P	1195.26 ± 280.27^{de}	2267.23 ± 80.41^{cd}
T1	0P	1309.91 ± 70.36^{d}	2113.59 ± 62.94^{d}
	50P	1552.89 ± 205.03^{c}	3183.22 ± 142.20^{b}
	100P	3735.73 ± 324.90^{a}	4115.84 ± 318.85a
	150P	2788.84 ± 164.25^{b}	3146.82 ± 55.23b
F-value		333.03	126.86
p-value		< 0.001	< 0.001

[(1)]Mean±standarderror.T1:With Inoculation of *B. diazoefficiens*;T0:without Inoculationof*B.diazoefficiens*;0 P:0 kgha^{-1} P_2O_5; 50 P:50 kgha^{-1} P_20_5;100 P:100 kgha^{-1}P_20_5;150 P:150 kgha^{-1}P_2O_5;AEZ:AgroEcologicalZone.
For each factor, same superscript letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the posthoc SNK test performed in case effects if the model were significant (p≤0.05).

equal and that recorded under the treatments T0+0P and T0+50P were statistically equal (Table 5).

Inoculation with *B. diazoefficiens* and phosphorus fertilization have a highly significant effect on total nitrogen yield relative to aboveground biomass (p <0.001) (Table 4). At Kétou the combination inoculated soybean combined to 100 kg ha^{-1} P_2O_5 allowed to have the best performance (59.19 kg ha^{-1}N) while the combination uninoculated combined to 0kg ha^{-1} of P_2O_5 gave the lowest yield. In Zè, the highest yield value was obtained with the combination inoculated soybean combined to 150 kg ha^{-1} P_2O_5 (96.96 kg ha^{-1} N) and the smallest value with the combination uninoculated combined to 0kg of P_2O_5 ha^{-1}. Finally, inoculation with *B. diazoefficiens* and phosphorus fertilization influence total nitrogen yield in grain in Kétou and Zè. The combination inoculated soybean combined to 100 kg ha^{-1} P_2O_5 allowed to have the highest total nitrogen yields in the grain which is respectively 144.13 kg ha^{-1} N and 295.02 kg ha^{-1} N at Kétou and Zè.

Effect of *B. diazoefficiens* inoculation and phosphorus fertilization on microbiological parameters of soybean

Nodule number was not significantly affected by the interaction (Inoculation with *B. diazoefficiens** phosphorus fertilization) at Kétou while at Zè, the nodule number was significantly affected by the interaction (Inoculation with *B. diazoefficiens** phosphorus fertilization). Overall, the inoculation with *B. diazoefficiens* had significantly increased the number of nodules for all of the phosphorus fertilizer amounts (Table 6). Those were 18.13; 26.94; 20.94; 22.69 nodules/plant for the treatments T1+0P; T1+50P; T1+100P; T1+150P compared with 0.13; 0; 0.06 and 0.25 nodules/plant respectively for the treatments T0+0P; T0+50P; T0+100P and T0+150P.

As for the mycorhization parameters, it was fund that the interaction (Inoculation with B. diazoefficiens* phosphorus fertilization) significantly mycorhization frequency ($p < 0.001$) and intensity ($p < 0.001$) only at Zè. The mycorhization frequency was significantly lower under the treatment T0+0P (41.46%) than the other treatments while that was higher under the treatment T1+50P (76.04%).

Likewise, the lowest mycorhization intensity was obtained under the treatment T0+0P (3.57%) and the highest was obtained under the treatment T1+50P (9.10%). In the site of Kétou, the interactive effect of Inoculation with *B. diazoefficiens* and phosphorus fertilization was not significant on the mycorhization parameters in both sites (Table 6).

Effect of *B. diazoefficiens* inoculation and phosphorus fertilization on the physicochemical parameters of soil

The combination of *B. diazoefficiens* inoculation and phosphorus application significantly influences all physicochemical parameters of soil (p <value <0.05) (Table 7). At Zè, the inoculated soybean combined to 50 kg ha^{-1} P_2O_5 gave the highest assimilation of phosphorus and the smallest with the uninoculated combined to 0 kg ha^{-1} P_2O_5. In Kétou, despite the existence of a significant difference, mean test could not differentiate the levels of combinations. Inoculation with *B. diazoefficiens* and phosphorus fertilization has no influence on pH. Furthermore, inoculation with *B. diazoefficiens* and phosphorus fertilization significantly influenced the nitrogen content only at Zè (p <value <0.001). However, the mean test could not differentiate the levels of combinations.

Finally, inoculation with *B. diazoefficiens* and phosphorus fertilization has a highly significant effect on organic carbon in only Zè. Inoculated soybean combined to 150 kg ha^{-1} P_2O_5 (T0P150) and Inoculated soybean combined to 100 kg (T0P100) resulted in the best organic carbon content (Table 7).

DISCUSSION

Effect of *B. diazoefficiens* inoculation and phosphorus application on agronomic parameters of soybean

The results showed that inoculation combined with phosphorus application improves soybean growth and yield. Height of the plants on the two sites at 45 days after sowing was the highest owing to the combination (inoculation * phosphorus fertilizer). This improvement in height is even more noticeable with *Bradyrhizobium* inoculation combined with 150 kg ha^{-1} P_2O_5 which resulted in height (43.82 cm at Kétou and 42.22 cm at Zè). These results are similar with those obtained by Fatima et al. (2006), Abbasi et al. (2010) and Zoundji et al. (2015) who showed that the combined application of phosphorus and *Bradyrhizobium* USDA 110 inoculation resulted in improved plant growth. This indicates that application of the different doses of phosphorus combined with inoculation with the *Bradyrhizobium* strain positively influences the physiological process and accelerates the growth process of the plant. Abbasi et al. (2010) reported stimulation of soybean growth following inoculation with *Bradyrhizobium* in nitrogen-deficient soil. Significant effects of inoculation combined with phosphorus application were observed on biomass yield (aboveground and root) and nitrogen yield in biomass and grain.

Biomass is an important indicator in the evolution of atmospheric nitrogen-fixing activity. According to Ballo et al. (2018), plants with high aboveground biomass would have a large root system. In the present study, the highest aboveground biomass yields were obtained with *B. diazoefficiens* inoculation combined with application of 100 kg ha^{-1} P_2O_5 in Kétou and 150 kg ha^{-1} P_2O_5 in Zè, as compared with the control (T0+0P). Similar trends were observed as far as the root biomass of soybean plants is concerned. This could be explained by the soil nitrogen content of 0.016% at Zè compared to 0.05% at Kétou before the experiment. These results are consistent with those obtained by Boulbaba et al. (2009) where, nitrogen fertilization-based *Bradyrhizobium* strains inoculation especially leads to an increase in biomass compared to the control. The results are also similar to those obtained by Muhammad (2010) which showed that the combination of inoculation with 50 kg P_2O_5 ha^{-1} significantly improves biomass production. Legumes such as soybeans require phosphorus for proper growth and nitrogen fixation (Drevon. 2017). Indeed, phosphorus is

Table 6. Effect of Bradyrhizobiumdiazo efficient strain and phosphorus supply on microbial parameters.

Treatment(1)		Nodulation		Mycorhization parameter (%)			
		Numberofnodules		Frequency (%)		Intensity (%)	
Ino	P	AEZ5 Kétou	AEZ6 Zè	AEZ5 Zè	AEZ6 Kétou	AEZ5 Zè	AEZ6 Kétou
T0	0P	6.13±2.93a	0.13±0.14b	41.46±0.91f	90.94±10.17a	3.57±0.2e	22.73±11.94a
	50P	5.81±4.17a	0±0b	64.58±2.94b	90.63±8.20a	4.37±0.30de	20.72±5.08a
	100P	6.63±2.79a	0.06±0.13b	60.83±3.39bcd	90±6.85a	5.93±0.61c	15.44±8.05a
	150P	9.19±4.22a	0.25±0.35b	60±0.89cd	94.69±2.95a	5.61±0.64cd	27.07±1.95a
T1	0P	16.50±10.8a	18.13±8.68a	63.12±01bc	95±4.45a	7.18±0.67b	27.20±3.6a
	50P	18.44±5.1a	26.94±12.51a	76.04±2.60a	94.38±4.62a	9.10±0.85a	20.90±10.44a
	100P	18.44±11.56a	20.94±6.08a	49.58±1.95e	91.8±3.89a	5.58±0.57cd	20.93±5.15a
	150P	19.38±3.91a	22.69±14.65a	58.71±2.64d	93.75±3.23a	5.62±0.53cd	25.97±8.67a
	F-value	3.52	9.59	83.82	0.922	27.31	0.44
	p-value	0.109	<0.001	<0.001	0.507	<0.001	0.863

(1)Mean ± standard error.T1:With Inoculation of Bdiazoefficiens; T0:without Inoculation of Bdiazoefficiens; 0P:0kgha^{-1} P_2O_5;50P:50 kgha-1P205;100P:100 kgha^{-1} P_2O_5;150P:150kgha-1P_2O_5;AEZ:AgroEcologicalZone.
For each factor, same super script letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the posthoc SNK test performed in case effects if the model were significant (p≤0.05).

Table 7. Effect of *Bradyrhizobium diazoefficiens* strain and phosphorus supply on soil physicochemical parameters.

Treatment (1)		Pass		pH(H2O)		N		CO	
Ino	P	AEZ5 Kétou	AEZ6 Zè	AEZ5 Kétou	AEZ6 Zè	AEZ5 Kétou	AEZ6 Zè	AEZ5 Kétou	AEZ6 Zè
T0	0P	31.96 ± 11.04b	30.43 ± 4.87c	5.68 ± 0.79a	6.4 ± 0.14a	0.05 ± 0.0d	0.06 ± 0.01a	0.63 ± 0.33a	0.47 ± 0.02b
	50P	35.61 ± 1.61b	35.39 ± 1.15bc	6.0 ± 0.32a	6.45 ± 0.1a	0.05 ± 0.01d	0.06 ± 0.01a	0.95 ± 0.54a	0.38 ± 0.03c
	100P	48.10 ± 6.31b	33.34 ± 2.93bc	5.750 ± 0.73a	6.68 ± 0.36a	0.08 ± 0.00c	0.06 ± 0.02a	0.61 ± 0.06a	0.62 ± 0.03a
	150P	67.33 ± 29.2ab	36.12 ± 3.46bc	5.63 ± 0.3a	6.57 ± 0.13a	0.10 ± 0a	0.07 ± 0.02a	0.73 ± 0.08a	0.60 ± 0.01a
T1	0P	36.84 ± 12.31b	33.94 ± 3.22bc	6.1 ± 0.48a	6.43 ± 0.15a	0.08 ± 0.01c	0.0 7± 0.02a	0.86 ± 0.29a	0.4 ± 0.04b
	50P	37.97 ± 24.27b	43.34 ± 2.06a	5.35 ± 0.56a	6.58 ± 0.19a	0.09 ± 0.01b	0.05 ± 0.01a	0.69 ± 0.07a	0.47 ± 0.06b
	100P	72.19 ± 15.6ab	38.64 ± 1.08b	5.85 ± 0.47a	6.45 ± 0.13a	0.09 ± 0.01b	0.06 ± 0.01a	0.75 ± 0.20a	0.44 ± 0.04b
	150P	84.02 ± 29.9a	33.22 ± 0.54bc	5.43 ± 0.4a	6.30 ± 0.14a	0.08 ± 0.01c	0.08 ± 0.02a	0.70 ± 0.20a	0.63 ± 0.03a
F-value		3.95	8.14	1.68	0.96	1.03	42.16	0.70	30.56
p-value		0.005	< 0.001	0.159	0.481	0.432	< 0.001	0.670	< 0.001

(1)Mean ± standard error.T1:With Inoculation of Bdiazoefficiens; T0:without tInoculation of Bdiazoefficiens; 0P:0 kgha^{-1} P_20_5; 50P:50 kgha^{-1} P_2O_5;100 P:100 kgha^{-1} P_2O_5;150P:150 kgha^{-1} P_2O_5; AEZ: Agro Ecological Zone. Pass: Available Phosphorus; pH: Soil pH water; N: Nitrogen; CO: Organic Carbon. For each factor, same super script letters denote no significant difference between the means at a given site. Different letters indicate significant differences of the posthoc SNK test performed incase effects if the model were significant (p≤0.05) one of the nutrients that play a key role in plant growth (Fernández et al., 2007). According to Ndakidemi (2014), it plays an important role in the use of sugar and starch, the use of photosynthesis, cell division and organization, the formation of nodules, root development, flower initiation and seed and fruit development. El-Ghandour et al. (1996) demonstrated that phosphorus fertilization resulted in better grain yield and high N content in soybean compared to the control. In addition, the author clarified that as P levels increased, the parameters studied also increased. According to Zoundji et al. (2015), the highest aboveground biomass yields obtained by inoculation and phosphorus supply are of utmost importance for improving soil fertility in a well-organized rotation system including legume crops, soybean for instance. Inoculation of *B. diazoefficiens* and phosphorus intake significantly improved nitrogen uptake by soybean in both aboveground and grain biomass at the three study sites.

The highest nitrogen yields in the aboveground and grains are observed with the inoculation and application of 100kg ha^{-1} P_2O_5, except aboveground biomass at Zè where inoculation combined with application of 150kg ha^{-1} P_2O_5 yielded the best result. These results confirm those of Tairo and Ndakidemi (2014) and Zoundji et al. (2015) who reported that the combination of inoculation and phosphorus application had a maximum positive effect on nitrogen uptake in soybeans. Also, the inoculation combined with 100kg P2O5 ha^{-1} application allowed to have the highest nitrogen contents in the grains at the three study sites.

According to Zoundji et al. (2015), better uptake of N by soybeans could directly benefit crops in extensive cropping systems in developing countries, where access to fertilizer producers is limited. According to Rahman et al. (2013), Nitrogen uptake by the biological fixation mechanism (BNF) by associated microbial populations is the main source of nitrogen for cereal crop production. Also, the type of soil can be at the origin of such differences, as it was suggested by Tellawi et al. (1986) and Boulbaba et al. (2009). These authors reported that this type of soil significantly affects nitrogen uptake, which could explain the difference observed at the two sites. Indeed, the richer the soil is in phosphorus and nitrogen, the more mycorhization inhibition is observed (Nagahashi et al., 1996). Similar results were obtained by Goudiaby et al. (2018) who showed the importance of having low phosphorus and nitrogen soils for better bradyrhizobium efficiency. Moreover, inoculation with *B. diazoefficiens* and phosphorus application significantly improved soybean grain yield at all two study sites. The inoculated soybean combined to 100 kg ha^{-1} P_2O_5 (inoculation + 100kg ha^{-1} P_2O_5) resulted in the highest yields of 4115.84 kg ha^{-1} against 1668.58 for the control in Zè and 1788.84 kg ha^{-1} against 901,40 kg ha^{-1} for the control in Kétou. The yields obtained in the current study are on average higher than those obtained by Abbasi et al. (2010) who obtained 2335kg ha^{-1} with 100kg ha^{-1} P_2O_5 and Zoundji et al. (2015) who obtained 2700kg ha^{-1} with 50kg ha^{-1} P_2O_5 application combined to inoculation with *Bradyrhizobium*.

These yields are nevertheless lower compared to the yields of 7610 kg ha^{-1} obtained by Akpalu et al. (2014) who used a dose of 139.4g per pot combined with inoculation with *B. diazoefficiens*. According to Ajakaiye (1980) and Sanginga et al. (1997), these results could have been attributed to the competitiveness of bacterial strains and their ability to make atmospheric nitrogen available to the plant. The results in the current work also showed that, regardless of the study site, the increase in phosphorus doses combined with inoculation with the *Bradyrhizobium diazoefficiens* USDA110 strain increased soybean grain yields. The increase in yield at Zè could be explained by the soil N content which is almost three times that of Kétou before the beginning of the experiment.

Effect of *B. diazoefficiens* inoculation and phosphorus fertilization on microbiological parameters of soybean

Inoculation of *B. diazoefficiens* and phosphorus application had a significant influence on all microbiological parameters of soybean ($p < 0.05$). The observed results on the number of nodules are similar to those obtained by Tran et al. (2007). Abbasi et al. (2010) and Zoundji et al. (2015) showed that application of *B. diazoefficiens* strain USDA110 + 60 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} P_2O_5, respectively, increased the number of nodules compared to the different conventional fertilizer methods used by producers. This could be due to the positive impact of phosphorus on the biofertilizer produced from *Bradyrhizobium* strain USDA110 which corresponds to better nitrogen nutrition of the plant.

The results in the current study showed that phosphorus did not affect the frequencies and intensities of mycorrhization regardless of the doses applied except for Ze where the Inoculation combined with application of 50 kg ha^{-1} P_2O_5 allowed the highest. These results are consistent with those obtained by Babajide et al. (2008) and Zoundji et al. (2015) who reported that mycorhization parameters may be slowing down when the soil phosphorus supply exceeds the plant's phosphorus requirements. Thus, when the amount of phosphorus added reaches a certain level, it has a negative effect on the microbiological parameters at the root level and inhibits the action of mycorrhizae which naturally allow the plant to draw phosphorus by developing its root system.

Effect of *B. diazoefficiens* inoculation and phosphorus fertilization on the physicochemical parameters of soybean

The combination of with *B. diazoefficiens* inoculation and phosphorus fertilization significantly influenced all physicochemical parameters of soil (p <value <0.05) at the two study sites. Thus, it has been noted an improvement in the phosphorus level, a decrease of both organic carbon and nitrogen available in the soil. These decreases in nitrogen levels could be explained by their high uptake by plants since in a soil moderately rich in nitrogen, the plant first starts by taking the available mineral nitrogen in the soil before the symbiotic fixation (Voisin et al., 2015). In fact, the nutrient requirement of soybean pushes it to explore its immediate environment and to draw the maximum resource for its growth (Meddich et al., 2017)

Conclusion

Inoculation with *B. diazoefficiens* USDA 110 strain combined with phosphorus application improved soybean growth and yield parameters. The best combination and dosage is inoculated soybeans combined with 100 kg ha^{-1} P_2O_5 (inoculation + 100 kg ha^{-1} P_2O_5). It has allowed a good accumulation of nitrogen in aerial and root biomass but also allowed the highest grain yields in soybeans.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abbasi KM, Muhammad M, Tahir MM (2010). Efficiency of rhizobium inoculation and P fertilization in enhancing nodulation, seed yield, and phosphorus use efficiency by field grown soybean under hilly region of Rawalakot Azad Jammu and Kashmir. Pakistan Journal of Nutrition 33(7):1080-1102.

Aganze MV (2014). Réponse de trois variétés de soja a l'inoculation par *Bradyrhizobium japonicum* sans limitation de phosphore et de potassium à buhehe/birava en territoire de Kabare (République Democratique du congo). Mémoire présenté en vue de l'obtention du Diplôme d'Ingénieur Agronome, 50p.

Ajakaiye CO (1980). Leaf nitrate reductase activity and crude protein in *Corchorus olitorius* and *Ceratotheeca sesamoides*. Nigerian Journal of Botany 1:36-42.

Akedrin AT, Voui BBNB, Akotto OF, Ake S (2020). Effets de six légumineuses spontanées les plus répandues dans les jachères naturelles sur la fertilité des sols dans la région de Daloa (Côte d'Ivoire). International Journal of Biological and Chemical Sciences. 14(3):1052-1064

Akpalu MM, Siewobr H, Oppong-Sekyere D, Akpalu SE (2014). Application and Rhizobia inoculation on growth and yield of soybean (*Glycine max* L. Merrill). American Journal of Experimental Agriculture 4(6):674-685.

Babajide PA, Akanbi WB, Alamu LO, Ewetola EA, Olatundji OO (2008). Growth, nodulation, biomass yield of soybean (*Glycine max L.*) as influence by bio-fertilizers under simulated eroded soil condition. Research Journal of Agronomy 2(4):96-100.

Ballo B, Turquin L, N'Gbesso M (2018). Effet de l'inoculum bactérien de la souche Irat–fa3 de B*radyrhizobium japonicum* sur la croissance et la nodulation de 3 variétés de soja cultivées en côte d'ivoire. Agronomie africaine 30(2):169-178.

AIEA (2020). Le Bénin augmente sa production et ses exportations de soja au moyen d'engrais biologiques et de la technologie isotopique. https://www.iaea.org/fr/newscenter/news/le-benin-augmente-sa-production-et-ses-exportations-de-soja-au-moyen-dengrais-biologiques-et-de-la-technologie-isotopique. Accessed on 08 July 2020.

Boulbaba L, Bouaziz S, Mainassara Z, Mokhtar H, Mokhtar L (2009). Effets de la fertilisation azotée, de l'inoculation par Rhizobium sp. et du régime des pluies sur la production de la biomasse et la teneur en azote du pois chiche, BASE [En ligne], 13(4):537-544. URL: https://popups.uliege.be:443/1780-4507/index.php?id=4745.

Drevon JJ (2017). Efficacité d'utilisation du phosphore pour la fixation symbiotique de l'azote et phytases des nodules de légumineuses. Innovations Agronomiques, INRA 60:3-10.

El-Ghandour IA, El-Sharawy MO, Abdel-moniem EM (1996). Impact of VAM fungi and rhizobium. The growth and P, N and Fe uptake by faba-bean. Fertilizer Research 43:43-48.

Fatima Z, Zia M, Chaudhary MF (2006). Effect of Rhizobium strains and phosphorus on growth of soybean (*Glycine max*) and survival of Rhizobium and P solubilizing bacteria. Pakistan Journal of Botany 38:459-464.

Fernández LA, Zalba P, Gómez MA, Sagardoy MA (2007). Activité de solubilisation des phosphates des souches bactériennes dans le sol et leur effet sur la croissance du soja en serre. Biology and Fertility of Soils 43(6):805-809.

Giller J, Dashiell KE (2007). *Glycine max* (L) Merill. Fiche de Protabase. Van der Vossen, H.A.M. & Mkamilo, G.S. (Editeurs). PROTA (Plant resources of Tropical Africa/Ressources Végétales de l'Afrique Tropicale), Wageningen, Pays Bas. Site :http : database.proba.org/recherche.htm. Accessed on 18 september 2018.

Giller KE (2001). Nitrogen fixation in tropical cropping systems. Second ed. CABI Publishing. Wallingford, UK P 448.

Goudiaby AO, Diedhiou S, Ndiaye S, Ndour N, Ndoye I (2018). Effet des substratssur lamycorhization et la croissance de Anacardium occidentale L.en pépinièreetdes sujets adultes sur lesparamètres physico-chimiques du sol. Afrique Science 14(6)148-159.

Graham PH, Rosas JC (1979). Phosphorus fertilization and symbiotic nitrogen fixation in common bean. Agronomy Journal 71:925-926.

Houngnandan P, Sanginga N, Woomer P, Vanlauwe B, Van Cleemput O (2001). Response of Mucuna pruriens to symbiotic nitrogen fixation by rhizobia following inoculation in farmers'fields in the derived savana of Benin. Biology and Fertility of Soils 33:416-422.

Index box (2019). Benin - Soya Beans - Market Analysis, Forecast, Size, Trends and Insights https://www.indexbox.io/store/benin-soya-beans-market-report-analysis-and-forecast-to-2025/

Jacobsen I (1985). The role of phosphorus in nitrogen fixation by young pea plants (*Pisum sativum*). *Physiologia Plantarium* 64:190-196.

Javaheri F, Baudoin GJH (2001). Le soja : 860-882. In : Raemaekers (Eds):Agriculture en Afrique Tropicale, DGCI, Bruxelles, Belgique 1634 p.

Kouelo AF, Badou A, Houngnandan P, Francisco MMF, Gnimassoun CJ-B, Sochime DJ (2012). Impact du travail du sol et de la fertilisation minérale sur la productivité de *Macrotyloma geocarpum* (Harms) Maréchal & Baudet au centre du Bénin. Journal of Applied Biosciences 51:3625-3632.

Kpenavoun S, Chogou FO, Santos F, Hounhouigan DJ (2018). Efficacité technique des producteurs de soja du Bénin. *Annales des Sciences Agronomiques* 22(1):12-35.

Meddich A, Mohamed AEM, Wahbi S, Boumezzough A (2017). Évaluation des potentialités mycorhizogènes en lien avec les paramètres physico-chimiques des sols de palmeraies du Maroc (Marrakech et Tafilalet). Cahiers Agricultures 26(4):45012

Muhammad A (2010). Response of a promiscuous soybean cultivar to rhizobial inoculation and phosphorus in Nigeria's Southern Guinea Savanna Alfisol, Nigerian Journal of Basic and Applied Sciences 18:79-82.

Nagahashi GDD, Douds JR, Abney GD (1996) "Phosphorus amendment inhibits hyphal branching of the VAM fungus (*Gigaspora margarita*) directly and indirectly through its effect on root exudation", Mycorrhiza 6(5):403-408.

Ndakidemi PA, Dakora FD, Nkonya EM, Ringo D, Mansoor H (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.

Ogumniyi S, Johan W, Andries J, Roem W (2012). Bacterial translocation and in vivo assessment of intestinal barrier permeability in rainbow trout (Oncorhynchus mykiss) 51 with and without soybean meal-induced inflammation. Journal of Nutritional Science (5):1-10.

Phillips JM, Hayman DS (1970). Improved procedures for cleaning roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions British. Mycological Society 55(1):93-130.

Plaxton WC (2004). Plant response to stress: Biochemical adaptations to P deficiency. Encyclopedia of Plant and Crop Science. Marcel Dekker, Inc., pp. 976-980.

PROGRAMME D'ACTIONS DU GOUVERNEMENT (PAG) (2016-2021). Présidence de la république du Bénin.

Rahman Z, Siddiqui MN, Khatun MA, Kamruzzaman M (2013). Effet de la farine de feuilles de goyave (Psidium guajava) sur les performances de production et la sensibilité aux antimicrobiens chez le poulet de chair commercial. Journal of Natural Products 6:177-187

Sanginga N, Dashiell K, Okogun JA, Thottalpilly G (1997). Evaluation of symbiotic properties and nitrogen contribution to of mucuna to maize grown in the derived savanna of West Africa. Plant and Soil 179:119-129.

Scherer HW, Pacyna S, Spoth K, Schulz M (2008). Low levels of ferredoxin, ATP and leghemoglobin contribute to limited N_2 fixation of peas (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) under S deficiency conditions. Biology and Fertility of Soils 44:909-916.

Schulze J, Temple G, Temple SJ, Beschow H, Vance CP (2006). Nitrogen fixation by white lupin under phosphorus deficiency. Annals of Botany 98:731-740.

Seyni B, Laouali IM (2020). Effets de la déficience en phosphore du sol sur la croissance et le développement du sésame (*Sesamum indicum L*.). Journal international des sciences biologiques et chimiques 14(3):1014-1024.

Waluyoa SH, Tek AL, Leendert't M (2004). Effect of phosphate on nodule primordia of soybean (*glycine max* merrill) in acid soils in rhizotron experiments. Indonesian Journal of Agricultural Science 5(2):37-44

Tairo EV, Ndakidemi PA (2014). Macronutrients Uptake in Soybean as Affected by *Bradyrhizobium japonicum* inoculation and phosphorus (P) supplements. American Journal of Plant Sciences 5:488-496.

Tellawi A, Haddad N, Hattar B (1986). Effect of several Rhizobium strains on nodulation, nitrogen uptake and yield of chick peas (*Cicer arietinum* L.). *Z. Pflanzenernaehr. Bodenkd*. 149:314-322.

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986). Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. Mycorrhizae. Physiology Genetics pp. 1-5.

Vieira RF, Mendes I, Res-Junior FB, Hungria M (2010). Symbiotic Nitrogen Fixation in Tropical Food Grain Legume: Current Status. Microbes for Legume Improvement 427 p.

Vincent JM (1970). A Manual for the Practical Study of Root-Nodule Bacteria. International Biological Programm Hanbook N°15. Blackwell Scientific Publications: Oxford, UK.

Voisin AS, Cellier P, Jeuffoy MH (2015). Fonctionnement de la symbiose fixatrice de N_2 des légumineuses à graines: Impacts Agronomiques et Environnementaux Innovations Agronomiques 43:139-160.

Zoundji CC, Houngnandan P, Amidou MH, Kouelo FA, Toukourou F (2015). Inoculation and phosphorus application effects on soybean [G*lycine max* (l.) Merrill] productivity grown in farmers' fields of Benin. Journal of Animal and Plant Sciences 25(5):1384-1392.