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INOCULATION AND PHOSPHORUS APPLICATION EFFECTS ON SOYBEAN [*Glycine max* (L.) Merrill] PRODUCTIVITY GROWN IN FARMERS' FIELDS OF BENIN

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ABSTRACT

Nitrogen deficiency is a major factor limiting crop production in most African agricultural soils. As legume, soybean can obtain a significant amount of its N requirement through symbiotic N_2 fixation when inoculated with effective and compatible *Bradyrhizobium* strains. An on farmer's fields' study was carried out in Northern and Centre Benin to d*et*ermine the effectiveness of *Bradyrhizobium japonicum* strains introduced in Benin cropping systems. Five inoculations treatments (control, FA3, STM3043, STM3045 and USDA110), two phosphorus levels (0 and 50 kg of P_2O_5 ha^{-1}) arranged in split plot design were established in twelve farmers' fields. Each farmer represented one replication. Results indicated that inoculation with different *Bradyrhizobia* strains improved significantly ($p < 0.001$ to $p < 0.05$) height, grain, biomass yield, nodulation, and nitrogen uptake of soybean but less than treatments where phosphorus application was combined to rhizobial inoculation. The most efficient strains identified were FA3 and STM3043 respectively in Northern and Centre Benin. In Northern Benin, the strain FA3 contributed to the increasing of 73% of the grain yield and 62% of the straw yield compared to the control, while in the Centre Benin the strain STM3043 was found to contribute to the increasing of 60% and 66% respectively of the grain and straw yields. Both strains FA3 and STM3043 could be used in cropping systems for improving soybean productivity in Benin.

Key words: Biofertilizer, cropping systems, nitrogen, yield, Benin.

INTRODUCTION

Most African tropical soils are degraded and characterized by low levels of soils nutrients and consequently by a continued decrease of crop yield (Saïdou *et al*., 2012). Indeed, nitrogen is the most limiting nutrient in agriculture (Van Cleemput *et al*., 2008). Improved soil fertility and agricultural productivity due to mineral fertilizer supply are widely studied (Morris *et al*., 2007). However, smallholder farmers in Sub-Saharan Africa are unable to afford the high costs of chemical fertilizers (Abaidoo *et al*., 2013). In addition, these chemical fertilizers are not always available and have residual effects on environment (Herridge *et al*., 2008; Adak *et al*., 2014). Aware of these undesirable situations, it is affordable to use farming practices that are inexpensive with fewer disadvantages, such as the use of legumes like soybean [*Glycine max* (L.) Merrill]. The crop can fix atmospheric nitrogen for the restoration and maintenance of soil fertility in a sustainable way and consequently could improve crop yields.

In fact, soybean is estimated to fix 80% of its nitrogen needs (Smaling *et al*., 2008). Soybean N_2-fixation is beneficial as it provide necessary N to the plant from the atmosphere which otherwise would proceed from soil and/or manure (Chianu *et al*., 2011). The fixation of soybean as much as 300 kg of N ha^{-1} in addition to the release in the soil, of 20 - 30 kg N ha^{-1} for the following crop had been estimated (Hungria *et al*., 2006).

To improve soybean yield, biological nitrogen fixation, contribution to soil fertility restoration, inoculation with efficient strains of *Bradyrhizobia* has already been tested in several countries (McInnes and Haq, 2007; Houngnandan *et al*., 2009; Afzal *et al.*, 2010); Hussain *et al*., 2011; Tairo and Ndakidemi, 2014).

Apart of nitrogen, phosphorus (P) is the second major plant growth-limiting nutrients in most agriculture soils (Shahid *et al.*, 2009). Phosphorus is quite abundant in soil but it reacts readily with iron, aluminium and calcium to form insoluble compounds, resulting in very low phosphorus availability (Zarrin *et al*., 2006). It plays an important role in the plant's energy transfer system since its deficiency r*et*ards growth (Shahid *et al.*, 2009). Symbiotic nitrogen fixation needed high phosphorus as large amounts of energy being consumed during the process of photosynthesis, or energy generating m*et*abolism depends on the availability of phosphorus (Schulze *et al*., 2006). Through its basic functions in plants as an energy source, phosphorus affects nodule development, production of protein, phospholipids and phytin in grains legume (Rahman *et al*., 2008). Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influence N fixation by legume plants (Abdul-Aziz, 2013). P-supplementation can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of macronutrients in legumes (Makoi *et al*., 2013).

There were several reports on the interaction b*et*ween *Rhizobia* inoculation and P supply. Ndakidemi *et al*. (2006) and Akpalu *et al*. (2014) had reported that combination of beneficial bacteria of soil and phosphorus in legume plants significantly increased nodulation, pod formation and development, and a subsequent grain yield comparatively to the single use of phosphorus or beneficial bacteria. The inoculation with *Rhizobium* and phosphorus supplementation improved the macronutrient uptake (N, P, K) in different organs of the whole plant of soybean (Tairo and Ndakidemi, 2014). Highly effective and comp*et*itive *Rhizobium* strains and a supply of appropriate amount of phosphorus (Scherer *et al*., 2008) could markedly increased legume growth and nitrogen fixation.

On station trial carried out in Benin (unpublished data) showed that phosphorous application at the dose of 50 kg P ha^{-1} and inoculation with *Bradyrhizobium* strain FA3 is affordable and could be appropriate for soybean production. In 2013 four *Bradyrhizobia* strains (FA3, STM3043, STM3045 and USDA110) were tested in farmers' fields of Benin. This study aimed to assess the effects of these *Bradyrhizobia* strains and phosphorus on soybean growth and yields in farmers' fields and to identify the most effective strains to be introduced in Benin cropping systems.

MATERIALS AND M*ET*HODS

Experimental site: The study was carried out in two Agro-Ecological Zones (AEZ) of Benin Republic from July to October 2013. The first being food-producing zone of southern Borgou (AEZ 3) is situated in Soudanian zone. This zone is located b*et*ween 1°10'-3°45' E and 9°45'- 12°25 N and is characterized by a unimodal rainfall with a mean annual rainfall less than 1000 mm. The relative humidity and temperature vary respectively from 18 to 99% and 24 to 31˚C. The mean temperature can reach 40˚C in March-April.

The second one (AEZ 5) being Cotton zone of central Benin is localized in Sudano-guinean zone situated b*et*ween 1°45'- 2°24' E and 6°25'- 7°30' N. The annual mean temperature is ranging b*et*ween 26°C and 29°C and the annual mean rainfall b*et*ween 1000 and 1400mm. The relative humidity varied from 69% and 97%.

Weather data obtained from the pan-African m*et*eorological service (ASECNA) of each study area are reported in table 1. In each zone, six farmer's fields were chosen for this study. In both zones, experiments were carried out in degraded ferruginous soils. Average soils characteristics of the farmer's fields are given in table 2.

One vari*et*y of soybeans (TGX 1910-14F) was tested. This vari*et*y had a maturity cycle of 100-120 days with potential grain yield of 4 t. ha^{-1}.

Four strains of *Bradyrhizobium japonicum* were used for inoculum production: FA3, STM3043 and STM3045 strains obtained from Laboratory of Mediterranean and Tropical Symbiosis of Montpellier (France) and USDA110 strain from Laboratory of Soils Microbiology of Nairobi University (Kenya).

Inoculum production: Each strain was cultivated separately in Yeast Extract-Mannitol-Agar medium (YEMA) as described by Vincent (1970) and was incubated for five days. Then, isolated colonies under growth on YEMA medium were transferred into Erlenmeyer flasks containing 300 ml of Yeast Extract-Mannitol (YEM) medium and placed under shaking conditions on a magn*et*ic stirrer for one week. A quantity of 20 ml of liquid culture, containing approximately 10^8 viable cells per milliliter, was thoroughly mixed with 80 g of neutralized and sterilized peat packaged in thermo-resistant polypropylene bags.

Experimental design: In each zone, experiments were conducted at six farmer's fields where maize was grown in the previous season in all fields in order to reduce soil N effects and soil h*et*erogeneity factor. A split plot design with two factors was considered. The main plot factor was inoculation with five variants (control, FA3, STM3043, STM3045 and USDA110) and the second factor was phosphorus with two levels (0 and 50 kg P_2O_5 ha^{-1}), making ten treatments in total. Each farmer represented one replication where the ten treatments were applied randomly. Plot receiving each treatment size was 10 m × 6 m and contained ten planting lines. The space b*et*ween rows was 75 cm and the inter-plant spacing was 15 cm. Five soybean seeds were sown per hole and later thinned to three, ten days after planting. Before sowing, soybean seeds were coated with *Bradyrhizobium* inoculants for inoculated treatments. One week after sowing, plots reserved for treatment with phosphorus received 50 kg ha^{-1} of P_2O_5 as tri super phosphate. A starter dose of 20 kg N ha^{-1} as urea was applied on all plots. All plots were weeded manually using hoe at three and six weeks after sowing.

Data collection: Two samplings were done during the study. Firstly, at 10 weeks after sowing (flowering time), a 1 m² quadrant was randomly placed in each plot. Soybean shoot and root were harvested separately in each quadrant. Before harvesting, height of ten soybean plants was measured in each plot. Then, a mean of 24 plants was harvested. Roots were washed and nodule were removed and counted. Thin fragments of root were cleared, stained and examined for Arbuscular Mycorrhizal Fungi (AMF) colonization. Shoot and nodules were oven dried at 65°C for 72 hours and their dry weight recorded. Nitrogen content of soybean shoot was analyzed by the Kjeldahl m*et*hod.

Secondly, at 17 weeks after sowing, a 9 m² quadrant was delimited for assessing, grain and straw yield. The number of pod was taken on ten plants in each plot. Nitrogen content of straw and grain was analyzed by the Kjeldahl m*et*hod. Straw/grain nitrogen uptake was d*et*ermined as N% x straw/grain yield.

Statistical analysis: All statistical analysis was carried out using SAS software version 9.2. One-way analysis of variance (ANOVA) was performed to d*et*ermine the statistical differences among the different strains of *Bradyrhizobium japonicum*. When significant differences ($p < 0.05$) were noticed, a Student-Newman-Keuls test was used to compare the means. The correlation b*et*ween nodule number, mycorrhizal colonization, biomass production, nitrogen uptake and grain yield of soybean was assessed by means of Pearson's correlation coefficient.

RESULTS

Symbiotic param*et*ers of soybean at ten weeks after sowing: Inoculation had a significant effect ($P < 0.05$; $p < 0.001$) on symbiotic param*et*ers of soybean in both zone except for nodule number which was not significant in AEZ 5 ($P > 0.05$). Phosphorus supplementation also significantly improved these param*et*ers in both zones except for AMF colonization rate which decreased with phosphorus supply (Table 3). In both zones, the control without phosphorus obtained the lowest values for nodulation param*et*ers. The combination of 50 kg of P_20_5 ha^{-1} to STM3043 strain gave the best nodule number and nodule dry weight in both zone (39 nodules plant^{-1} and 497 mg plant^{-1} in AEZ 3 and 29 nodules plant^{-1} and 366 mg plant^{-1} in AEZ 5). The lowest averages were for the control (14 nodules plant^{-1} and 120 mg plant^{-1} in AEZ 3, 13 nodules plant^{-1} and 111 mg plant^{-1} in AEZ 5). The four strains of *Bradyrhizobium japonicum* induced more nodulation in soybean root in AEZ 3 than AEZ 5. About AMF colonization rate, STM3045 strain without phosphorus supply produced the highest average in both zone (62% in AEZ 5 and 61% in AEZ 3).

Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on the height and shoot dry weight of soybean at ten weeks after sowing: Significant differences were found b*et*ween *Bradyrhizobium japonicum* strains ($p<0.05$) for height and shoot dry weight of soybean at ten weeks after sowing (Table 4) in both zones. Phosphorus supply also significantly influenced these param*et*ers. In AEZ 3, STM3043 strain combined to 50 kg of P_20_5 ha^{-1} had the maximum height (93 cm plant^{-1}) with a gain of 52% compared to the control. For the variable shoot dry weight, the highest value was found with FA3 strain + 50 kg of P_20_5 ha^{-1} (22.2 g per plant).

In AEZ 5, the height of soybean plants varied from 36 cm (control + 0 kg of P_20_5 ha^{-1}) to 88 cm (STM3043 + 50 kg of P_20_5 ha^{-1}). The highest shoot dry weight was 23 g plant^{-1} (STM3045 + 50 kg of P_20_5 ha^{-1}). The lowest value was found with control without P (6 g plant^{-1}).

Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on the number of pods, straw yield and grain yield of the soybean at seventeen weeks after sowing: Results in table 5 showed significant effects of *Bradyrhizobium japonicum* strains and phosphorus supply on number of pods, straw yield and grain yield in AEZ 3 and AEZ 5.

In AEZ 3, the best performances were observed with plots receiving FA3+ 50 kg of P_20_5 ha^{-1}. The average values found in this case were 91 pods plant^{-1}, 5274 kg ha^{-1} of straw and 2739 kg ha^{-1} of grains when the control without phosphorus produced only 38 pods plant^{-1}, 1810 kg ha^{-1} of straw and 770 kg ha^{-1} of grain.

The same trends were observed in AEZ 5 for these param*et*ers but the best values were found with treatment STM3043 strain + 50 kg of P_20_5 ha^{-1}. It recorded 82 pods plant^{-1}, 4439 kg ha^{-1} and 2711 kg ha^{-1} respectively for straw and grain yields.

Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on Nitrogen uptake of soybean: Inoculation and phosphorous application significantly improved N uptake of soybean (Table 6). The highest shoot N uptake and grain N uptake were obtained with the strains FA3 and STM 3043 respectively in AEZ 3 and AEZ 5. In AEZ 3 at 10 WAS and 17 WAS, the shoot N uptake varied from 31 kg N ha^{-1} to 86 kg N ha^{-1} and from 17 kg N ha^{-1} to 68 kg N ha^{-1} respectively. The grain N uptake ranged b*et*ween 53 kg N ha^{-1} and 133 kg N ha^{-1}. Likewise, in AEZ 5, the shoot N uptake varied from 35 kg N ha^{-1} to 77 kg N ha^{-1} and from 19 kg N ha^{-1} to 54 N kg ha^{-1} at 10 WAS and 17 WAS respectively. The grain N uptake ranged b*et*ween 27 kg N ha^{-1} and 135 kg N ha^{-1}

Table 1. Weather data of study areas.

Parameters	**Agro-Ecological Zones**	**July**	**August**	**September**	**October**
Tmax (°C)	AEZ 3	29.5	28.1	29.8	30.2
	AEZ 5	30.5	29.6	31.1	32.2
Tmin (°C)	AEZ 3	21.7	21.1	21.5	21.7
	AEZ 5	22.2	22.1	22.4	22.7
RHmax (%)	AEZ 3	94	95	95	94
	AEZ 5	93	93	91	92
RHmin (%)	AEZ 3	66	67	64	62
	AEZ 5	65	64	61	62
Total rainfall (mm)	AEZ 3	139.2	148.6	260.5	134.3
	AEZ 5	101.5	98.5	125.5	80.2

Tmax: monthly average maximum temperature; Tmin: monthly average minimum temperature; RHmax: monthly average maximum relative humidity; RHmin: monthly average minimum relative humidity

Table 2. Physico-chemical properties of the soil before the starting of the experiment.

Zones	**pH (H_2O)**	**N (%)**	**C (%)**	**OM (%)**	**Total P (ppm)**	**Available P (ppm)**	**CEC (meq/100g)**	**Sand (%)**	**Silt (%)**	**Clay (%)**
AEZ 3	6.9	0.06	1.7	2.9	89.9	32.2	15.9	76.2	11.0	12.2
AEZ 5	6.4	0.04	1.1	1.8	91.3	29.8	12.0	81.4	8.8	8.7

N: Nitrogen; C: Carbon; OM: Organic Matter; P: Phosphorus; CEC: Cation Exchange Capacity

Table 3. Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on symbiotic parameters of soybean at ten weeks after sowing.

***Bradyrhizobium* strains**	**Phosphorus (P)**	**Nodule number ($plant^{-1}$)**		**Nodule dry weight (mg $plant^{-1}$)**		**Mychorization rate (%)**	
		AEZ 3	**AEZ 5**	**AEZ 3**	**AEZ 5**	**AEZ 3**	**AEZ 5**
Control	50P	20a	20a	161a	168a	22b	23b
	0P	8b	5b	79b	54b	40a	41a
	Mean	**14B**	**13A**	**120B**	**111B**	**31A**	**32A**
FA3	50P	25a	24a	320a	264a	30b	31b
	0P	15b	10b	136b	103b	52a	53a
	Mean	**20AB**	**17A**	**228B**	**184AB**	**41CB**	**42CD**
STM3043	50P	39a	29a	497a	366a	26b	27b
	0P	17b	9b	177b	117b	46a	47a
	Mean	**28A**	**19A**	**337A**	**242A**	**36C**	**37D**
STM3045	50P	26a	25a	250a	213a	36b	37b
	0P	14b	11b	113b	92b	61a	62a
	Mean	**20AB**	**18A**	**182B**	**152AB**	**49B**	**50B**
USDA110	50P	24a	19a	287a	241a	34b	35b
	0P	16b	10b	137b	92b	59a	60a
	Mean	**21AB**	**15A**	**212B**	**166AB**	**47B**	**48CB**
Pr		$0,0111^{*}$	$0,0373^{ns}$	$0,0015^{**}$	$0,0013^{**}$	$<0,0001^{***}$	$<0,0001^{***}$
CV		54	46	40	61	20	19

Means followed by a same letter with same character in the same column are not significantly different at $p < 0.05$ according to Student – Newman-Keuls test. ns: Not significant; * : significant (p‹5 %) ** : Significant (p ‹1 %) ; *** : Significant (p‹1 ‰)

Table 4: Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on the height, shoot dry weight and nitrogen yield of soybean at ten weeks after.

Bradyrhizobium strains	Phosphorus (P)	Height (cm)		Shoot dry weight (g plant^{-1})	
		AEZ 3	AEZ 5	AEZ 3	AEZ 5
Control	50P	72a	73a	11a	12a
	0P	41b	36b	8a	6b
	Mean	**56B**	**54B**	**10B**	**9B**
FA3	50P	79a	73a	22a	19a
	0P	59b	63b	10b	10b
	Mean	**69A**	**68AB**	**16A**	**14A**
STM3043	50P	93a	88a	21a	21a
	0P	70b	62b	11b	11b
	Mean	**82A**	**75A**	**16A**	**16A**
STM3045	50P	83a	72a	17a	23a
	0P	70b	57b	12b	10b
	Mean	**76A**	**65AB**	**15A**	**16A**
USDA110	50P	90a	75a	15a	20a
	0P	69b	58b	11a	11b
	Mean	**79A**	**66AB**	**13A**	**16A**
Pr		0.013**	0.021*	0.064ns	0.016*
CV		9	24	43	45

Means followed by a same letter with same character in the same column are not significantly different at $p < 0.05$ according to Student – Newman-Keuls test. ns: Not significant; * : significant (p‹5 %) ** : Significant (p ‹1 %) ; *** : Significant (p‹1 ‰)

Table 5. Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on the number of pods, straw yield and grain yield of the soybean at seventeen weeks after sowing.

Bradyrhizobium strains	Phosphorus	Pods number (plant^{-1})		Straw yield (kg ha^{-1})		Grain yield (kg ha^{-1})	
		AEZ 3	AEZ 5	AEZ 3	AEZ 5	AEZ 3	AEZ 5
Control	50P	62a	60a	3547a	2512a	1757a	1864a
	0P	38b	28b	1810b	1679b	770b	863b
	Mean	**50B**	**44B**	**2679B**	**2096B**	**1264B**	**1363B**
FA3	50P	85a	68a	5274a	3191a	2739a	2428a
	0P	62b	49b	2813b	2418b	1633b	1363b
	Mean	**73A**	**59A**	**4344A**	**2805AB**	**2186A**	**1901AB**
STM3043	50P	91a	75a	4786a	4439a	2327a	2711a
	0P	55b	54b	3370a	2537b	1289b	1663b
	Mean	**73A**	**65A**	**4078A**	**3488A**	**1808AB**	**2187A**
STM3045	50P	71a	82a	3820a	3693a	2464a	2341a
	0P	49b	51b	3156a	2753b	1509b	1393b
	Mean	**60AB**	**66A**	**3488A**	**3223A**	**1986A**	**1867AB**
USDA110	50P	71a	72a	3594a	2828a	2160a	2397a
	0P	49b	53b	2896a	2757b	1124b	1478b
	Mean	**60AB**	**62A**	**3245A**	**3292A**	**1642AB**	**1938AB**
Pr		0.0012**	0.0056**	0.0304*	0.0033**	<0.0001***	0.0084**
CV		18	28	44	33	15	31

Means followed by a same letter with same character in the same column are not significantly different at $p < 0.05$ according to Student – Newman-Keuls test. ns: not significant; * : significant (p‹5 %) ** : Significant (p ‹1 %) ; *** : Significant (p‹1 ‰)

Table 6. Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on N uptake of soybean.

Bradyrhizobium strains	Phosphorus	Shoot N uptake at 10 WAS (kg N ha^{-1})		Shoot N uptake at 17 WAS (kg N ha^{-1})		Grain N uptake at 17 WAS (kg N ha^{-1})	
		AEZ 3	AEZ 5	AEZ 3	AEZ 5	AEZ 3	AEZ 5
Control	50P	54a	45a	24a	29a	88a	84a
	0P	31b	35b	17b	19b	53b	27b
	Mean	**43B**	**40B**	**21B**	**24B**	**71B**	**56B**
FA3	50P	86a	62a	68a	37a	133a	121a
	0P	41b	45b	28b	25b	89b	80b
	Mean	**64A**	**53A**	**44A**	**31AB**	**111A**	**101AB**
STM3043	50P	82a	77a	61a	55a	125a	135a
	0P	46b	49b	30b	29b	86b	92b
	Mean	**64A**	**63A**	**46A**	**43A**	**106A**	**144A**
STM3045	50P	70a	69a	45a	43a	115a	109a
	0P	45b	43b	30b	29b	80b	76b
	Mean	**57A**	**56A**	**38AB**	**36AB**	**98AB**	**93B**
USDA110	50P	70a	71a	44a	44a	110a	130a
	0P	45b	41b	29b	26b	81b	74b
	Mean	**58A**	**56A**	**37AB**	**35AB**	**96AB**	**102AB**
Pr		**0,039****	**0,033****	0.006**	0.004**	0.021*	0.005**
CV		11	15	8	10	15	21

Means followed by a same letter with same character in the same column are not significantly different at $p < 0.05$ according to Student – Newman-Keuls test. ns: not significant; * : significant (p‹5 %) ** : Significant (p ‹1 %) ; *** : Significant (p‹1 ‰).

Table 7. Coefficient of correlation between nodule number, mycorrhizal colonization, biomass production, nitrogen yield and grain yield of soybean.

Parameters	**Nodule number**	**Mycorrhizal colonization**	**Biomass production**	**Shoot N uptake**	**Grain yield**	**Grain N uptake**
Nodule number	-	0.71**	0.86**	0.93**	0.90***	0.91**
Mycorrhizal colonization	0.71**	-	0.68**	0.61**	0.58**	0.63**
Biomass production	0.86**	0.68**	-	0.78***	0.71*	0.75***
Nitrogen uptake	0.93**	0.61**	0.78**	-	0.63**	0.93**
Grain yield	0.90***	0.58**	0.71*	0.63**	-	0.72**
Grain N uptake	0.91**	0.63**	0.75**	0.93	0.72**	-

* : significant (p‹5 %) ** : Significant (p ‹1 %); *** : Significant (p‹1 ‰)

DISCUSSION

Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on symbiotic parameters of soybean: Rhizobial inoculation increased significantly both the number and dry weight of nodules. Similar results were obtained by Zhang *et al*. (2011) and Abbasi *et al*. (2010) who reported that inoculation of soybean significantly increased the nodule number over the control. Phosphorus supply also improved nodule number and nodule dry weight. This result corroborates that obtained by Tran *et al*. (2007), who showed that the application of *Bradyrhizobium japonicum* strain USDA110 + 60 kg of P_2O_5 enhanced the number of nodule and nodule dry weight compared to the various conventional fertilization methods used by producers. Tekle and Walelign (2014) reported that phosphorous fertilizer significantly improved nodule number per plant and nodule dry weight of soybean and supposed that the reason might be the overall performance of the plant improved by phosphorous leading to a better nodulation. A significant and positive correlation was found between nodule number, biomass production, nitrogen uptake and grain yield (Table 7). So, increases of nodule number involve better nitrogen nutrition which provided a good development of shoot and root of the plant and improved grain yield (Sharma *et al.*, 2011). However, poor performances of nodulation were obtained in some fields in both zones. Those poor performances could be due to the development in the soil of endogenous *Bradyrhizobium* strains which sometimes have antagonistic effects on new strains introduced as stated by Mathu *et al*., 2012 whose work has shown that inoculation in soils where endogenous rhizobia strains are strongly present (more than 10^3 bacteria g^{-1} of soil) is often unsuccessful. This has justified the presence of nodules on the control.

Inoculation positively affected mycorrhizal colonization and results in table 7 showed that mycorrhizal colonization was positively correlated with nodule number. Similar results were found by Onguene *al*. (2012) on four promiscuous soybean vari*et*ies in Cameroon. On the contrary, AMF colonization rate dropped considerably with phosphorus supply. This could be due to a property of mycorrhizal fungi whose roots colonisation is associated with low levels of soil fertility (especially phosphorus) as well reported by Babajide *et al*. (2008). Willmann *et al.* (2013) reported that mycorrhizal development may be precluded when soil P supply exceeds P requirements of the crop. So, threshold levels of soil solution, phosphorus that limit mycorrhizal development should not be exceeded in order to improve arbuscular mycorrhizal association. But, in spite of the importance of P proved in this study, access to P fertilizer could also be a problem for smallholder farmers because of its cost and unavailability on the mark*et*. In such a situation, exploitation of arbuscular mycorrhizal fungi would be affordable and may greatly improve the P supply to the host plant by increasing the absorbing capacity of the roots.

Effect of *Bradyrhizobium japonicum* strains and phosphorus supply on soybean growth and yield: The inoculation improved the height of soybean plants compared to the control. This improvement is even more important when P is combined to Bradyrhizobial inoculation. A similar result was obtained by Afzal *et al.* (2010) who indicated that inoculation with TAL 377 strain of *B. japonicum* induced an increase of the height of soybean plants.

A positive effect of inoculation and phosphorus was also observed on the number of pods, straw and grain yields of soybean with means significantly higher than the control. Thus, the highest grain yields were 2739 kg ha^{-1} (FA3 + 50 kg of P_20_5 ha^{-1}in) AEZ 3 and 2711 kg ha^{-1} (STM3043 + 50 kg of P_20_5 ha^{-1}in) AEZ 5 while the yield of controls (non-inoculated without phosphorus) was around 800 kg ha^{-1}. The average grain yield of soybean under the inoculation and phosphorus supply were lower compared to yields of 7610 kg ha^{-1} obtained by Akpalu *et al*. (2014). However, the yields obtained were alike the average soybean yields (550-2200 kg.ha^{-1}) as shown by Javaheri and Baudouin (2003).

The same trends were noted on the yield of straw and the number of pods per plant which are directly related to grain yield. Similar results were found by Muhammad (2010) who showed that the combination of IRj 2180A rhizobial strain to 50 kg of P_20_5 ha^{-1} significantly increased shoot biomass and number of pods over those of the control. The best yields of straw obtained with inoculation and phosphorus intake are of utmost importance for improving soil fertility in a well organized crop rotation system. Straw could also be used as fodder to feed animals.

The *B. japonicum* inoculation and phosphorus supply improved nitrogen uptake by soybean. Thus, the yield was significantly higher on the inoculated treatments than those uninoculated. This can be explained by a good symbiotic activity induced by the different strains of Rhizobium. These results confirm those of Tairo and Ndakidemi (2014) who indicated that the combination of phosphorus and inoculation showed maximum positive effect on nitrogen uptake in soybean. Improved N uptake by soybean could directly benefit crops in extensive cropping systems in developing countries, where access to fertilizer farmers is limited. Indeed, the greater N uptake in the biomass permits to assess the economy in nitrogen fertilizer for succeeding crops. Rahman *et al*. (2014) reported that soil N supply through biological nitrogen fixation (BNF) by associated microbial populations is the principal source of N for cereal crop production. Likewise, the nitrogen uptake could affect the crude protein and crude fiber of fodders as stated by Khogali *et al*. (2011). However, there was a variation of the nitrogen uptake according to different strains of *B. japonicum* studied. Indeed, this reflects a difference in the quality of symbiotic relationships implemented by different strains of Rhizobium with soybeans.

Effect of study areas on the response of soybean to rhizobial inoculation and phosphorus supply: There was a variation in the response of soybean inoculation and phosphorus according to the two study areas. The best nodulation, height, number of pod, N uptake straw yield and grain yield were obtained in AEZ 3. This could be due to the variability of environmental conditions b*et*ween the two studies, but also to the physico-chemical and biological characteristics of soils as reported by Vriezen *et al*. (2007). Indeed, weather data especially moisture conditions were more favorable in AEZ 3 than in AEZ 5 (Table 1). In addition to m*et*eorological conditions, basic information about chemical characteristics of soils (Table 2) showed that soils of AEZ 3 had b*et*ter nutrients contents (nitrogen, carbon, organic matter, available phosphorus, Cation Exchange Capacity) than those of AEZ 5.

The best strains were FA3 in AEZ 3 and STM3043 in AEZ 5. So, STM3043 strain could be able to improve soybean productivity in arid conditions considering that it rained more in AEZ 3 than in AEZ 5 during experiments. The presence of *Rhizobia* able to improve legume productivity under unfavorable condition is very interesting, because these *Rhizobia* represent the best source of nitrogen especially in arid and semi-arid regions, where they contribute to land stabilization and fertilization (Lebrazi *et al*., 2014).

Conclusion: The inoculation with *Bradyrhizobium japonicum* strains and phosphorus supply improved productivity of soybean in Benin. The use of these effective strains of *Rhizobia* and phosphorus supplementation could be an effective way to enhance the growth of soybean and consequently nutrients uptake. However, phosphorus supplementation could be reduced by a co-inoculation of soybean with *Mycorrhizae* and *Rhizobia*.

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