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Effect of different phosphorus rate combined to rhizobium biofertilizers on Bambara groundnut [*Vigna subterranea* (L.) Verdcourt] productivity in Benin

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Abstract

Rhizobial inoculation and phosphorus supplementation could be an alternative means of supplying important nutrients in legumes crops for their higher yields. This study was carried out to determine the optimal rate of phosphorus in combination with *Rhizobium* for Bambara groundnut improvement. Experiments were carried out in Northen Benin and in Central Benin. Five levels of phosphorus (0 kg, 20 kg, 40 kg, 60 kg and 80 P ha^{-1}) and two levels of inoculation (inoculation with Rhizobium strain and no inoculation) were arranged in Randomized Complete Block design repeated five times. Results showed that inoculated treatments were significantly higher than non-inoculated treatment. Phosphorus supplementation significantly influenced the nodulation, shoot and root dry weight, straw and grain yield and nitrogen uptake of Bambara groundnut in both areas. The inoculation combined with the 60 or 80 kg ha^{-1} rate of phosphorus induced a 62% and 46% increase in grain and straw yield in the Centre and 78% and 58% increase in straw yield compared to the yields obtained on control in northern Benin. N uptake varied from 12.26 to 60.28 kg N ha^{-1} in the Centre and from 13.99 to 61.2 kg N ha^{-1} in the northern Benin. No significant difference was not found between the treatments combined inoculation and application of 60 or 80 kg ha^{-1} of phosphorus. So, application of 60 kg ha^{-1} of phosphorus fertilizer combined to inoculation with a suitable Rhizobia strain could be considered as an alternative by small farmers for increasing productivity of Bambara groundnut.

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Introduction

Bambara groundnut [*Vigna subterranea* (L.) verdc.] is one of the most important seed legumes in sub-Saharan Africa, particularly in Benin (Gbaguidi *et al.*, 2015). Adapted to a variety of climatic and ecological conditions (Mbosso *et al.*, 2020), this plant is mainly cultivated for its importance in the food balance for populations, especially in rural areas (Bamshaiye *et al.*, 2011), its well-balanced chemical composition of carbohydrate 63%, protein 19% and fat 6.5% (Mkandawire, 2007; Mbaiogaou *et al.*, 2013), its economic and social importance (Gbaguidi *et al.*, 2015). It is also a good source of vitamins and minerals (calcium, magnesium, and potassium). Bambara groundnut is less demanding in terms of water and grows easily on marginal land where it where it provides substantial income (Konate *et al.*, 2019). Like all legumes, Bambara groundnut contributes to soil fertilization, through the symbiotic fixation of fixation of atmospheric nitrogen when it is associated to efficient Rhizobium bacteria (Mbosso *et al.*, 2020).

Despite all efforts made to improve Bambara groundnut yield, its production remains under negative influence of many constraints. One of the most important of them is soil infertility and environmental pollution (Gbaguidi *et al.*, 2015). Although as a legume Bambara groundnut can contribute to improving soil fertility through nitrogen fixation, the absence of effective rhizobium strains in its rhizosphere may limit this nitrogen-fixing ability (Ouoba *et al.*, 2016). To address this issue of soil infertility and environmental pollution, the use of organic fertilizers microbial biofertilizers such as rhizobia and mycorrhiza (Ngakou *et al.*, 2012) have been prioritized and applied nowadays (Ngakou *et al.*, 2014; Ridine *et al.*, 2014). Some authors showed the role of rhizobium inoculation in improvement of Bambara groundnut productivity (Gomoung *et al.*, 2017; Ikenganiya *et al.*, 2017).

Otherwise, research proved that nodulated legumes need more phosphorus than non-symbiotic plants that grows solely on a mineral nitrogen source. Indeed, the direct relationship between nitrogen fixation and phosphorus content on nodules simply proves how important phosphorus is to legumes (Rotaru and Sinclair, 2009, Cabeza *et al.*, 2014). N2-fixing legumes that are grown with inadequate phosphorus did not grow well because nitrogen fixation on bacteroid, ammonium absorption of amino acids and ureides in the plant cell of nodules are not enough to support plant growth (Hernandez *et al.*, 2009). These processes require more phosphorus in the transfer of energy which occurs in nodule functioning (Rotaru and Sinclair, 2009). In addition to this, the phosphorus required for mitochondrial and symbiosomal membrane synthesis during nodule development increases N2-fixing legume's demand for phosphorus even more (Rotaru and Sinclair, 2009). Ikenganiya *et al.* (2017) found that inoculation of Bambara groundnut associated with phosphorus application positively influenced its growth and productivity in Nigeria. Abbasi *et al.* (2010), Akpalu *et al.* (2014) reported that the 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 or beneficial nutrients. 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).

The current study aims to determine the optimal rate of phosphorus in combination with *Rhizobium* inoculation for Bambara groundnut improvement in two contrasted areas of Benin.

Material and methods

Study areas

Experiments were carried out in Northen Benin at Natitingou and in Central Benin at Ouèssè. Natitingou is situated in West Atacora zone (Agro-Ecological Zones 4) in Soudanian zone. This zone is located between 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.

Ouèssè is localized in Cotton zone of central Benin (Agro-Ecological Zones 5) in Sudano-guinean zone situated between 1°45’- 2°24’ E and 6°25’- 7°30’ N. The annual mean temperature is ranging between 26°C and 29°C and the annual mean rainfall between 1000 and 1400mm. The relative humidity varied from 69% and 97%.

Physico-chemical properties of the soil before the starting of the experiment were reported in Table 1. In the North, the soil was sandy-loam texture, slightly acid (6.29), had a good Nitrogen (N) content (0.13%), low organic matter content and moderate Phosphorus (P) content (10.51 ppm). In the centre, the soil was sandy-loam texture and moderately acid (5.95), had a good N content (0.12%), very low organic matter content and moderate P content (10.51 ppm).

Table 1. Physico-chemical properties of the soil before the starting of the experiment

Parameters	Zones	
	North	Centre
pH (H_2O)	6.29	5.95
N(%)	0.13	0.12
C (%)	0.87	0.47
OM (%)	1.5	0.81
Available P (ppm)	10.51	9.8
Sand (%)	68.1	70.4
Silt (%)	12.08	9.97
Clay (%)	17.31	18.9

Experimental design

Five farmer’s fields were considered per study areas and each farmer’s field was a repeat of the experimental design. Plots were arranged as a Randomized Complete Block repeated five times. Treatments were made from a combination of two factors. Five levels of phosphorus (0 kg, 20 kg, 40 kg, 60 kg and 80 P ha^{-1}) in the form of super phosphate triple and two levels of inoculation (inoculation with Rhizobium strains and without inoculation) were used. Each plot was contained ten planting lines. The space between rows was 50 cm and the inter-plant spacing 20 cm LMSEM 338 Rhizobia strain used in this study was obtain from Soil microbiology and microbial Ecology Laboratory. One variety of Bambara groundnut (Beige color) was selected for the study. Four seeds were sown per hole and later thinned to three 14 days after. The different rate of P_2O_5 (phosphorus) was applied on all plot 14 days after sowing.

Data collection

Two samplings were done during study. At flowering stage, shoots and roots of Bambara groundnut were separated. Roots were washed to remove adhering soil. Nodules were removed, counted, and dry weight taken. Shoots and roots were dried at 65°C for 72 hours and ground. Nitrogen content was analyzed by the Kjeldahl method (Sahelemedhin and Taye, 2000). At maturity stage, straws and grains were sampled on an area of 3 m x 2 m per plot, dried and weighed. The total N in the tissue samples were quantitatively determined by Kjeldahl procedure. Biological nitrogen fixation was expressed from the nitrogen accumulated using a polynomial regression: $Y = 0.0127X2 - 0.5354X + 17.441$ where Y represents the biological nitrogen fixation and X the amount of nitrogen accumulated per hectare (Bado *et al.*, 2018).

Statistical analysis

Data were compiled on a spreadsheet, inspected and then summary statistics calculated. One-way analysis of variance (ANOVA) performed using SAS software to determine the statistical differences among the different treatments. When significant differences ($p < 0.05$) were noticed, a Student-Newman-Keuls test was used to compare the means.

Results and discussion

Effect of phosphorus and inoculation on nodulation (nodule number and nodule dry weight) of Bambara groundnut

The results of the analysis of variance showed that inoculation and phosphorus significantly influenced the nodulation of Bambara groundnut in central and northern Benin. In central and northern Benin, inoculation combined with the application of the 60 or 80 kg ha^{-1} of phosphorus rate resulted in the highest nodule number compared to plots without inoculation and phosphorus input, which had the lowest nodule numbers (8.9 and 10.31 nodule/plant). There was no significant difference in nodule numbers per plant for treatments combining inoculation with 60 and 80 kg ha^{-1} of phosphorus. The same trend was obtained for the effect of inoculation and phosphorus on nodule dry weight (Table 2). The highest nodule dry weights were obtained on plots inoculated with 60 and 80 kg ha^{-1} of Phosphorus (85.2 and 85.34 mg $plant^{-1}$ respectively). The positive results from this study could be attributed to the efficiency of the Rhizobium strain which stimulated growth of the assessed parameters. So biofertilizers technology is an efficient supplier of nitrogen in the tested legume and it could replace the chemical nitrogen fertilizers. Similar to our study, significant improvement of effect of legume yield and yield components due to rhizobia inoculation was also documented by Maresha *et al.* (2017) and Ballo *et al.* (2018).

Effect of phosphorus and inoculation on height, shoot and root dry weight of Bambara groundnut

The results of the analysis of variance of the effect of the inoculation combined with the different phosphorus rates are presented in Table 3. It appears that inoculation combined with different doses of phosphorus significantly influenced the height, shoot dry weight and dry weight of Bambara groundnut roots in central and northern Benin. Inoculation of Bambara groundnut and application of 60 or 80 kg ha^{-1} of phosphorus significantly increased all assessed growth parameters (height, shoot dry weight and root dry weight). For all parameters the effect increased with the phosphorus dose. Height varied from 12.97 cm to 31.82 cm in the Centre and from 13.10 cm to 32.94 in the Northern Benin. Shoot dry weight ranged between 3.02 g $plant^{-1}$ and 4.98 g $plant^{-1}$ in the Centre and from 3.23 g $plant^{-1}$ and 5.26 g $plant^{-1}$ in the North. Values of root dry weight were between 1.20 and 3.97 g $plant^{-1}$ in the Centre and 1.44 and 4.05 g $plant^{-1}$ in the North.

Table 2. Effect of phosphorus and inoculation on nodulation (nodule number and nodule dry weight) of Bambara groundnut

Inoculation	Phosphorus	Nodule number (Number $plant^{-1}$)		Nodule dry weight (mg $plant^{-1}$)	
		Centre	North	Centre	North
	0	8.9d	10.31c	24.22d	26.35d
	20	12.73cd	13.27c	28.51c	30.52c
Uninoculated	40	17.84b	19.57b	46.94b	56.34ab
	60	24.40ab	25.51ab	69.66ab	69.94ab
	80	25.3ab	25.98ab	70.1ab	70.21ab
	0	15.25c	16.54bc	29.23c	31.54c
	20	18.85b	19.52b	49.80b	54.57b
Inoculated	40	25.45ab	26.84ab	75.88ab	79.83ab
	60	32.24a	33.15a	81.56a	85.2a
	80	33.21a	34.5a	82.2a	85.34a
Cv		20.00	22.42	47.53	28.47
Probability		< 0.0001	< 0.0001	0.0152	< 0.0012

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.

The increase in height and in the number of leaves per plant observed in the Bambara groundnut following inoculation with rhizobium and combined at different doses can be explained by the efficiency of the rhizobium strain and the role of phosphorus in the production of the energy necessary for photosynthesis. Indeed, according to N'Gbesso *et al.* (2010), when faced with an efficient strain of bacteria, the morphological and physiological parameters of a compatible variety are improved. This is due to the symbiotic nitrogen fixation provided by a large population of bacteria brought to the soil by inoculation. Studies conducted by Gomoung *et al.* (2017) and Ikenganiya *et al.* (2017) show that inoculation of Bambara groundnut with rhizobium bacteria combined with phosphorus improved their growth.

Table 3. Effect of phosphorus and inoculation on height, shoot and root dry weight of Bambara groundnut

Inoculation	Phosphorus	Height (cm)		Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})	
		Centre	North	Centre	North	Centre	North
Uninoculated	0	12.97d	13.10d	3.02d	3.23c	1.20d	1.44d
	20	17.99c	18.61c	3.29c	3.41c	1.96c	2.03cd
	40	22.38c	23.96c	3.44c	3.69b	2.46c	2.63b
	60	25.80b	26.41b	3.85b	3.89a	2.80bc	2.94b
	80	26.3b	27.01b	3.90b	4.01	2.98b	3.02b
Inoculated	0	13.82d	15.12d	3.52c	3.77b	2.05c	2.73c
	20	21.81c	22.18c	3.93b	3.93a	2.83b	3.01b
	40	26.71b	27.92b	4.21ab	4.84ab	3.24ab	3.58ab
	60	31.21a	32.01a	4.85a	5.01a	3.67a	3.86a
	80	31.82a	32.94a	4.98a	5.26a	3.97a	4.05a
Cv		8,67	3.84	4.30	8.04	8.56	12.88
Probability		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

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.

Table 4. Effect of phosphorus and inoculation on grain and straw yield of Bambara groundnut

Inoculation	Phosphorus	Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})	
		Centre	North	Centre	North
Uninoculated	0	499.70e	698.93f	775.95e	864.41e
	20	848.24d	902.60d	1021.95d	1191.91c
	40	1028.19c	1035.54c	1267.25c	1281.81b
	60	1299.78b	1307.52b	1323.01b	1397.26b
	80	1321.32b	1335.21b	1345.03b	1419.38b
Inoculated	0	766.75d	818.02^{e}	893.62e	972.59d
	20	1031.27c	1092.99c	1205.25c	1257.50c
	40	1127.98b	1235.25b	1324.21b	1452.39b
	60	1325.82a	1548.35a	1451.35a	1502.78a
	80	1398.71a	1597.37a	1487.94a	1531.97a
Cv		20.17	13.91	22.80	8.08
Probability		0.0078	< 0.0001	0.0054	< 0.0001

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.

Table 5. Effect of phosphorus and inoculation on N uptake and amount of N fixed of Bambara groundnut

Inoculation	Phosphorus	N uptake (kg N ha^{-1})		Amount of N fixed (kg N ha^{-1})	
		Centre	North	Centre	North
Uninoculated	0	12.26f	13.99f	11.79e	12.65d
	20	15.49e	15.21^{e}	13.27d	13.86c
	40	16.89d	17.50d	14.73c	14.97b
	60	18.78c	19.87d	15.18c	16.26b
	80	19.25c	20.24c	18.35b	18.67
Inoculated	0	16.17d	18.28d	12.03e	12.85d
	20	19.69c	19.80c	13.72a	14,21b
	40	32.34b	34.26b	15.34c	16.35b
	60	59.61a	60.21a	32.29a	33.10a
	80	60.28a	61.2a	32.98a	33.87a
Cv		26.54	12.49	2.17	13.19
Probability		0.0216	0.0151	< 0.0001	< 0.0004

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.

Biomass is an important indicator in the evolution of atmospheric nitrogen-fixing activity. Inoculation with rhizobium strains significantly improved the above-ground and root biomass per plant. Treatments that produced high above-ground biomass have a significant root system. Since inoculation is a catalyst for fertilization to improve biomass, the response of the plants would be explained by the amount of nitrogen supplied by the inoculation to cover the plants' needs. Our results are like those reported by Njeru *et al.* (2020). Similarly, Muhammad *et al.* (2010) and Zoundji *et al.* (2015) reported respectively on groundnut and soybean that inoculation with rhizobium strains significantly influenced the above-ground and root biomass of each specimen.

Effect of phosphorus and inoculation on grain and straw yield of Bambara groundnut

The combination of phosphorus application and inoculation had a significant effect on the yield parameters of Bambara groundnut (grain and straw yields) in central and northern Benin. The treatments combined inoculation and application of 60 or 80 kg ha^{-1} of phosphorus resulted in the highest grain and straw yields (Table 4). The inoculation combined with the 60 or 80 kg ha^{-1} rate of phosphorus induced a 62% and 46% increase in grain and straw yield in the Centre and a 78% and 58% increase in straw yield compared to the yields obtained on the uninoculated plots that received no phosphorus in northern Benin.

This result can be explained by the fact that the high presence of rhizobium bacteria in the soil reinforced biological nitrogen fixation. The phosphorus applied plays an important role in photosynthesis. High photosynthetic activity results in the production of a large amount of biomass and consequently an increase in grain yield. N'Gbesso *et al.* (2017) and Njeru *et al.* (2020) observed the same results by studying the effect of inoculation with several rhizobium strains on several leguminous varieties.

Effect of phosphorus and inoculation on N uptake and amount of N fixed of Bamabara groundnut

The results of the analysis of variance of the effect of inoculation and phosphorus on Nitrogen uptake and amount of Nitrogen fixed (Table 5) reveal that in both regions, the combination of inoculation and phosphorus significantly influenced Nitrogen uptake (respectively $p = 0.0216$ in the Centre and $p = 0.0151$ in the north) and amount of Nitrogen fixed ($p < 0.0001$). Treatments combining inoculation with 60 and 80 kg ha^{-1} of phosphorus gave the best nitrogen uptake and fixed nitrogen. However, nitrogen uptake and fixed nitrogen did not differ statistically between the treatments combined inoculation and application of 60 or 80 kg ha^{-1} of phosphorus. N uptake varied from 12.26 to 60.28 kg N ha^{-1} in the Centre and from 13.99 to 61.2 kg N ha^{-1} in the northern Benin. Amount of N fixed ranged between 11.79 and 32.98 kg N ha^{-1} in Centre and between 12.65 and 33.87 kg N ha^{-1} in the Northern Benin.

The increase in nitrogen yield is the consequence of the perfect symbiosis between the variety and the strain used. Nitrogen accumulation is beneficial in that it allows non-nitrogen-fixing crops to benefit from the nitrogen reserve. As a result, the supply of nitrogen in the form of mineral fertilizer would be reduced, thus contributing to the improvement of soil fertility. The results obtained during this study corroborate with those obtained by Asante *et al.* (2020) showed that inoculation significantly influences the amount of N accumulated in the biomass of Bambara groundnut plants. Also, Islam *et al.* (2017) and Houngnandan *et al.* (2020) showed that inoculation significantly improves the nitrogen yield of soybean biomass. Yakubu *et al.* (2010) reported similar results showing that nitrogen content increased by inoculation by 39% on groundnut control on sandy soil in Sudan. Biological N fixation by legumes is an indicator of their potential contribution to N recycling in cropping systems (Bado *et al.*, 2018). The increase in the amount of nitrogen fixed in Bambara groundnut plants following inoculation combined with phosphorus input is explained by the strong symbiotic activity between the bacteria and the plant facilitated by the phosphorus input. The low yield obtained in the control can be explained by the presence of the indigenous strains, which are less abundant and less efficient.

Conclusion

The results of this study showed that there was an interaction between rhizobium inoculation and phosphorus application. Inoculation combined to phosphorus increased nodulation, growth and yield parameters, nitrogen uptake and amount of nitrogen derived from atmosphere of Bambara groundnut. However, no significant difference was not found between the treatments combined inoculation and application of 60 or 80 kg ha^{-1} of phosphorus. Based on the above findings, application of 60 kg ha^{-1} of phosphorus fertilizer combined to inoculation with a suitable Rhizobia strain could be considered as an alternative by small farmers for increasing productivity of Bambara groundnut.

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