



## CROP SCIENCE

# Do biofertilizers affect nodulation ability and pod production in peanut genotypes?

ANA KELLY S. JULIÃO, LUCAS N. DA LUZ, MARYSSOL T. GADELHA, MATHEUS L. OLIVEIRA, MARIA V.S. SILVEIRA, SAMUEL F.A.O. CASTRO & LENIN P. BARROS

**Abstract:** Peanuts are an important legume for the Northeastern Brazilian market, but their production in this region is low. The present study aimed to evaluate the effect of biofertilizer doses on peanut nodulation and production components, to define the best dose and genotype. The experiment was conducted in a completely randomized block design (DBC) in a 3 x 3 factorial arrangement, with three replications. The treatments consisted of two evaluation factors: 1) Fertilization via different doses of organic fertilizer applied to the substrate (D1 = 0mL; D2 = 500mL; and D3 = 1000mL); and 2) Peanut genotypes (BR-1, UNI43 and UNI08). The following traits were assessed: number of nodules per plant (NNP), number of mature pods (NVM), pod mass per plant (MVP), seed mass per plant (MSP) and root length (CR). Peanut production is affected by fertilization via bovine biofertilizer, mainly for the components NVM, MVP and MSP. Besides, nodulation is a likely conditioner. The 1000mL dose proved to be the best treatment for the traits analyzed, and UNI08 accession, the most responsive material.

**Key words:** *Arachis hypogaea* L., *Rhizobium*, semiarid, oilseeds.

## INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a legume of the Fabaceae family capable of establishing symbiosis with bacteria of the orders Rhizobiales and Burkholderiales (Moreira & Siqueira 2006). The crop plays an important economic role for producers, with wide edaphoclimatic adaptation and nutritional / industrial versatility in northeastern Brazil, due to its biochemical composition (Jongrungklang et al. 2011). However, due to the lack of sufficient inputs and mechanization, the productive performance of the crop in the region is low (Aquino et al. 2013).

In the scenario of climate change, partly caused by the use of fossil fuels and fertilizers (Costa et al. 2016), organic fertilizers play an important role in the development of ecologically based agriculture. Among these fertilizers, the

biofertilizer stands out, which is a bioactive input that benefits development, gas exchange, nutrient extraction and symbiotic relationships in plants (Sousa et al. 2013, Viana et al. 2013).

Soil fertility is fundamental for the improvement of crop performance. In peanut cultivation, inoculants and nutrients with different formulations are strategies used to achieve high grain yield (Vieira 2011, Sousa et al. 2013, Souza et al. 2019). However, the use of chemical fertilizers with high concentrations of nitrogen in their formulations may decrease or inhibit inoculation (Reis et al. 2000).

Studies developed by Empresa Brasileira de Pesquisa Agropecuária Oeste (2000) show that Tatu peanut plants, inoculated and supplied with P and K, have a 25% higher seed yield, compared to plants inoculated and supplied with N, P and K; and 36,5% higher than plants supplied

with N, P and K, without inoculation. Thus, the lack of nutrients in the agricultural system may decrease peanut production efficiency and jeopardize inoculation results. Probably due to the inhibitory effect of nodulation and biological fixation.

Since producers do not feel secure to use N fertilizers, besides nodulation inhibition, biofertilizers emerge as an excellent alternative. For Bucher & Reis (2008), the application of biofertilizers may affect the survival of the fixing bacteria, due to their high water retention capacity, nontoxicity and water solubility. In addition, they provide N, P and K without affecting FBN (Biological Nitrogen Fixation).

The Northeast region is considered the second largest consumer market in Brazil (Mari et al. 2013), where production is mainly conducted by family farmers, with low use of technologies. As a strategy to improve crop productivity in Brazil semi-arid region, Santos et al. (2005) suggest rhizobial inoculation in soils where the number of these microorganisms is insufficient to guarantee nodulation above 70%.

However, it is known that the increased rhizobia quantity and functionality depend on abiotic conditions (Gualter et al. 2008), soil fertility, including iron and molybdenum availability and co-factors in the activation of the atmospheric N<sub>2</sub> fixation reaction. Therefore, FBN optimization in tropical species is subject to a joint selection of factors, such as the genetic variability of symbionts and the ability to respond to interaction. Thus, this study aimed to evaluate the effect of biofertilizer doses on peanut nodulation and production components and define the best dose and genotype.

## MATERIALS AND METHODS

The work was conducted on Fazenda Experimental Piroás, in pots under full sun, from July to September 2018. The experimental area belongs to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), located in the Maciço do Baturité, in the municipality of Redenção – Ceará state (4°14'53"S; 38°45'10"W and altitude of 340 m). According to Köppen, the climate of the region is characterized as tropical rainy Aw (Köppen 1923).

The seeds were selected from the UNILAB germplasm bank. One cultivar was used as a control (BR-1) and two as accessions (UNI43 and UNI08). Three seeds were planted in each 11-liter plastic pot. Twenty days after sowing (DAS), thinning was conducted, and one plant was left in each pot. Irrigation was performed by watering at the coldest hours of the day, at the end of the day, so as to avoid evaporation.

The three peanut genotypes of the *Fastigiata* subspecies were used. The accession UNI43 belongs to the *Spanish* group, while the other genotypes are part of the *Valencia* group.

The experiment was conducted in a completely randomized block design (DBC), in a combination of the level of two factors under evaluation (3 x 3), being 9 treatments and three replications. The treatments consisted of two evaluation factors: 1) Fertilization via different doses of organic fertilizer applied to the substrate (D1 = 0mL; D2 = 500mL; and D3 = 1000mL); and 2) Peanut genotypes (BR-1, UNI43 and UNI08).

Soil from the area was used as substrate, for the establishment of the experiment (on the farm), together with sand, in a 2: 1 ratio. The samples were collected and sent to the laboratory, to assess the chemical and physical conditions of the substrate. The analyses were performed according to the methodology described by Silva (1999), as characterized in Table I.

The bovine biofertilizer was prepared from a mixture of equal parts (1:1) of fresh bovine manure (0,05 m<sup>3</sup> of bovine excrement) and non-saline water (0,05 m<sup>3</sup>) (CEa = 0.8 dS m<sup>-1</sup>), under aerobic fermentation, for 30 days in a 100 liter (0,1 m<sup>3</sup>) plastic container. The applications were conducted in two different occasions: vegetative phase and flowering. The counts started after the total emergence of the stand, which occurred 15 days after sowing (DAS).

Table II shows the chemical analysis of the biofertilizer with its respective nutrient contents (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn).

The maximum recommendation of chemical adduction was adopted to meet the nutritional requirements of peanuts, provided by Fernandes (1993), corresponding to: 15 kg ha<sup>-1</sup> of N, 62.5 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 50 kg ha<sup>-1</sup> of K<sub>2</sub>O. As a reference, for a stand of 15,000 plants, the maximum dosage per plant<sup>-1</sup> in the cycle would be: 1 g N; 4.2 g P<sub>2</sub>O<sub>5</sub> and 3.3 g K<sub>2</sub>O.

The amount of nutrients found in the substrate was calculated as it follows: based on the multiplication of soil density (1,3) by the volume of soil placed in each pot (11 L), a value of 14.3 kg of soil per pot was obtained. Then, it was multiplied by the amounts of N, P and K to obtain

the amount of nutrients present in the substrate and the need for nutritional supplementation (Table III).

During the production cycle: 1 L of biofertilizer was employed, and the doses were provided in two 500mL applications (vegetative stage and flowering).

Therefore, 2/3 of the experiment received a concentration of 500mL, ten days after total emergence (D2 = 500mL), and 1/3 of the stand received another 500mL dose, at the 25<sup>th</sup> day, totaling 1000ml (D3 = 1000mL). The solution was sieved before application and measured in graduated containers.

At 90 DAS, the following traits were evaluated: number of nodules per plant (NNP) - obtained by direct counting; number of mature pods (NVM); pod mass per plant (MVP); seed mass per plant (MSP) and root length (CR).

The data were eventually submitted to analysis of variance (ANOVA), at 5% probability. When the F test was significant for the main effects and interaction, the treatment averages were compared by the Tukey test, at 5% probability. It was used the statistical software system ASSISTAT 7.7.

**Table I. Chemical and physical analysis of the substrate used before genotype cultivation and biofertilizer application, Redenção, Ceará, 2018.**

Chemical traits												
MO	N	P	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	H <sup>+</sup> + Al <sup>3+</sup>	Al	SB	CTC	CEes	pH
g kg <sup>-1</sup>	g kg <sup>-1</sup>	kg <sup>-1</sup>	cmol <sub>c</sub> kg <sup>-1</sup>									dS m <sup>-1</sup>
16.96	0.92	0.18	0.12	2.7	2.1	0.03	1.82	0.05	5.1	7.0	0.23	6.0
Physical traits												
Sand			Silt			Clay			Density			
----- % -----									g cm <sup>-3</sup>			
61			10			25			1,3			

MO – Organic matter; N – Nitrogen; P – Phosphorus; K<sup>+</sup> – Potassium; Ca<sup>2+</sup> – Calcium; Mg<sup>2+</sup> – Magnesium; Na<sup>+</sup> – Sodium; H<sup>+</sup> + Al<sup>3+</sup> – Hydrogen + Aluminum; Al – Aluminus; SB – Sum of bases (Ca<sup>2+</sup> + Mg<sup>2+</sup> + Na<sup>+</sup> + K<sup>+</sup>); CTC – Cation exchange capacity – [Ca<sup>2+</sup> + Mg<sup>2+</sup> + Na<sup>+</sup> + K<sup>+</sup> + (H<sup>+</sup> + Al<sup>3+</sup>)]; the pH was measured in aqueous extract (1: 2,5). CEes – electrical conductivity of the saturated extract of soil.

**Table II.** Chemical traits of the liquid biofertilizer applied to the soil, Redenção, Ceará, 2018.

Biofertilizer	Components										
	N	P	K	Ca	Mg	S	Fe	Cu	Zn	Mn	
Bovine	----- g L <sup>-1</sup> -----							----- mg L <sup>-1</sup> -----			
	2.73	1.7	1.6	3.1	0.6	-	42.6	0.2	6.1	6.1	

N – Nitrogen; P – Phosphorus; K – Potassium; Ca – Calcium; Mg – Magnesium; S – Sulphur; Fe – Iron; Cu – Copper; Zn – Zinc; Mn – Manganese.

**Table III.** Estimate of nutrient supply by substrate and nutritional supplementation needs, Redenção, Ceará, 2018.

Chemical characteristics	Nutrient		
	N	P	K
Recommendation	(g planta <sup>-1</sup> )		
	1	4.2	3.3
Substrate	(g kg <sup>-1</sup> )		
	0.92	0.18	0.12
	(14.3 kg planta <sup>-1</sup> )		
	13.15	2.57	1.72
Need for nutritional supplementation	(g planta <sup>-1</sup> )		
	0	1.63	1.58
Need for organic fertilizer for supplementation with biofertilizer	(L planta <sup>-1</sup> )		
	1	1	1

N – Nitrogen; P – Phosphorus; K – Potassium.

## RESULTS AND DISCUSSION

Genotypes and biofertilizer concentrations affected nodulation and agronomic components of peanut crop (Table IV). On the other hand, the interaction between these factors significantly affected only the agronomic trait number of mature pods (NVM). Regarding the coefficients of variation, there were oscillations between 8.22 and 20.31%, which are values considered average and satisfactory for experiments in vases, according to Pimentel (2009).

Peanut genotypes showed response variability in number of nodules per plant by the F test at 1% significance (Figure 1). It is possible to verify that the accession UNI08 presented better nodulation, followed by access UNI43 and cultivar BR-1, respectively. It is observed that the genotype

UNI08 has almost twice as many nodules as the roots of the control cultivar BR-1.

These results can be explained by the genetic components intrinsic to the inoculation process involving genotypes and groups of native rhizobial strains, mainly including the hormone group-dependent molecular signals and association specificity levels, via the selection of symbiont pairs (Zilli et al. 2006, Lima et al. 2021, 2022).

Santos et al. (2005) studied the effectiveness of isolated rhizobia from Northeastern soils in peanut cultivars and found that cultivar BR1 generally formed nodules with less mass and ineffective, due to the lack of the typical color of effective nodulation, compared to the other cultivars.

The regulation of control of N<sub>2</sub> fixation and nodulation, in different diazotrophic associated with plant species, alternates with the key

**Table IV.** Summary of variance analysis for number of nodules per plant (NNP), number of mature pods (NVM), pod mass per plant (MVP), seed mass per plant (MSP) and root length (CR) of three peanut genotypes submitted to different biofertilizer doses, Redenção, Ceará, 2018.

FV	GL	QM				
		NNP	NVM	MVP	MSP	CR
<b>Genotypes (G)</b>	2	62878.74**	3945.99**	1839.40**	881.95**	429.29**
<b>Doses (D)</b>	2	100972.76**	4676.02**	986.20**	463.93**	899.71**
<b>G/D</b>	4	2469.63 <sub>ns</sub>	2891.34**	87.81 <sub>ns</sub>	375.52 <sub>ns</sub>	59.27 <sub>ns</sub>
<b>Residue</b>	18	110.41	51.76	54.77	48.42	26.05
<b>CV (%)</b>		8.22	13.96	14.16	20.31	9.70
<b>M</b>		577.40	51.54	52.26	85.76	52.61

QM – Medium square; ns – non-Significant; \*\* – Significant at 5% significance by the F test; CV – Coefficient of variation; FV – Source of variation; GL – Degrees of freedom; M – Overall average.

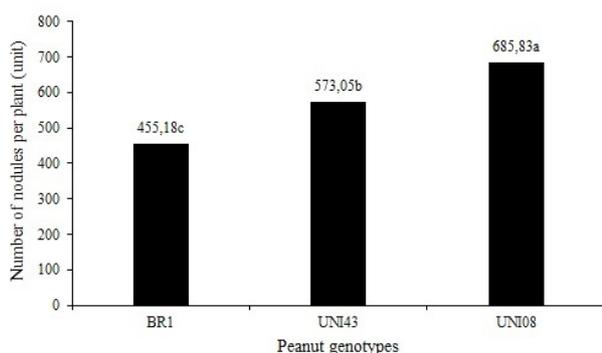
regulatory proteins and coevolution networks established between symbionts (Dixon & Kahn 2004). Thus, nodulation capacity and efficiency in cultures may vary within the same genus or species (Doyle & Luckow 2003).

In peanuts, nodulous strains have already been identified as non-nodular species, a phenomenon linked to Nod + and Nod- nodulation genes, respectively (Gorbet & Burton 1979). Changes in Nod genes cause disturbances in the infection process, such as the inability to form the infection cord, which results in the formation of empty, non-fixing nodules, a phenotype defined as Nod-.

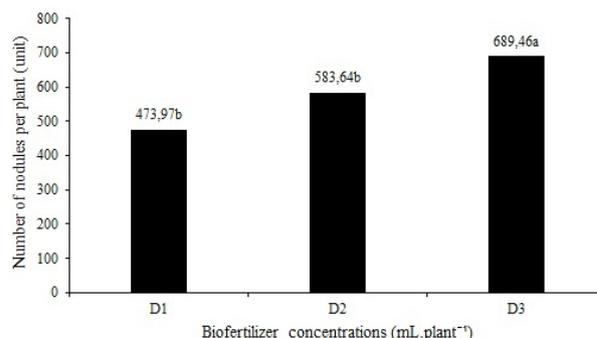
In their studies on inoculation with nodulous (Nod +) and non-nodulous (Nod-) peanut

lineages, Peng et al. (2018) observed that nodules and root hairs were absent in Nod- plants and present in Nod + lines. Nod- plants were shorter, with low tillering, yellow leaves and fewer pods per plant, compared to Nod + strains.

Concerning the effect of biofertilizer concentrations on NNP, there was a statistical difference between the applied treatments by the F test at 1% significance (Figure 2). Besides, D3 was able to increase the number of peanut nodules by up to 105.82, compared to D2. When his value is compared to the treatment without fertilizer application, it is noted that the addition of fertilizer in its highest concentration increased the nodules in the plants by 215.49.



**Figure 1.** Comparison of the number of nodules in the roots of peanut genotypes, Redenção, Ceará, 2018. Equal letters do not differ statistically by the F test at 1% significance.



**Figure 2.** Biofertilizer doses affecting the number of nodules in the roots of peanut plants, Redenção, Ceará, 2018. Equal letters do not differ statistically by the F test at 1% significance.

This type of response may increase traits of crop yield, such as number of pods and seeds. Thus, a nutritional enhancement via FBN (Souza et al. 2019) and the availability of other nutrients through biofertilizers are necessary to ensure more efficient and optimized peanut grain yield, as well as decreased crop cycle.

Benicio et al. (2012) investigated the effects of biofertilizers and modes of application on cowpea nodulation and found that the use of organic fertilizers via soil increases the number of nodules per plant and dry matter of the nodules of the culture.

Since cattle-based manure inputs are major inductors of the elevation of these traits, biofertilizers are expected to have directly affected the association by changing the concentration of macronutrients and micronutrients (Mantilla et al. 2010) such as P and K, which favor nodulation (Krolow et al. 2004).

Regarding the comparison of means of the number of mature pods for genotypes and concentrations (Table V), in D1 the UNI43 and UNI08 treatments are statistically equal and different from Access BR1 cultivar, in D3 all accesses differed between themselves, however the UNI08 access showed the highest average number of pods.

Isolatedly, within each treatment, only accession UNI08 responded to the applied concentrations, and D3 induced the best results.

The same effect was found when the genotypes in treatment D3 were compared. The material UNI08 presented the highest rate for the number of mature pods formed from the application of 1000mL concentration.

Such results can be explained by the specific genetic responsiveness of the accessions studied. Thus, there is positive selectivity for the increased production components (Luz et al. 2010) when subjected to exogenous nutrition sources, such as biofertilizers.

Therefore, while assessing the productive performance of the peanut crop under doses of castor bean cake and goat manure, Leite et al. (2015) came to the result that both the doses and the sources contributed significantly to the increase of the production components of the species, including the number of pods per plant and pod mass. Such evidence reveals positive responses to the use of organic fertilization in the crop.

According to Santos et al. (2009) the number of pods per plant in BR1 peanut ranges from 12 to 22 pods, which is lower than the results of the related research for the application of treatments with bovine biofertilizers.

Since nitrogen is an essential element for protein production and an indispensable constituent for the initial synthesis of embryos during seed germination (Falcão Neto et al. 2011) the addition of sources of this nutrient

**Table V. Means for number of mature pods (NVM) of three peanut genotypes submitted to different concentrations of bovine biofertilizer, Redenção, Ceará, 2018.**

Doses	NVM		
	BR1	UNI43	UNI08
D1	22.52aB	46.66aA	40.83bA
D2	34.83aA	48.66aA	37.00bA
D3	34.60aC	59.00aB	69.75aA

BR-1 – cultivar control; UNI43 and UNI08 – peanut access. Means followed by the same letter do not differ from each other by the Tukey test ( $p \leq 0.05$ ). Horizontal uppercase letters are comparing genotypes, and the vertical lowercase letters are comparing treatments. D1 – No biofertilizer application, D2 – 500mL application, D3 – 1000mL application.

establishes a positive relationship in the formation, production and filling of the pods for grain production in peanut culture.

Regarding the results of the response of each genotype to the trait pod mass per plant, the accessions showed greater response effect when compared to the commercial variety (Figure 3). Accession UNI08 increased the weight of its pods by 87% when compared to cultivar BR-1, and the values obtained were 62.25g and 35.88g, respectively.

These differences can be attributed to the genetic constitution of the accessions (Fachin et al. 2014) which determine the productive potential and the number of reproductive structures, as observed by Luz et al. (2010) in the relationship between the number of pods per plant and the number of total pegs in different peanut genotypes.

Between the treatments applied only the treatments D3 (Figure 4) showed a significant difference from the other treatments, presenting a higher average for pod mass gain. The increased peanut pod mass may be related to seed formation and filling. Such condition requires greater nutrient availability in the soil. To meet this nutritional need, the C/N ratio of the organic material was 10.8.

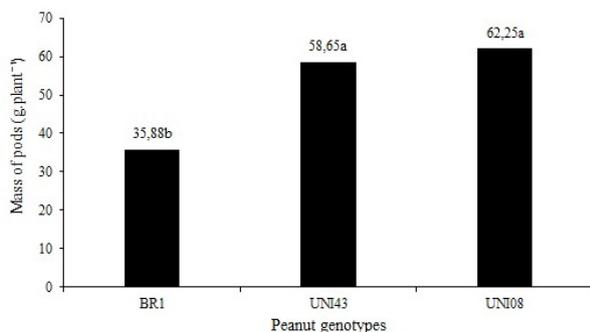
Since the substrate presented insufficient phosphorus availability for peanuts, based on the recommendation, this nutrient may have been made available via biofertilizer because the amount applied at the highest dose meets the need of the crop. This increased phosphorus availability will improve nodulation efficiency due to higher plant ATP availability (Silva 2010).

Regarding genotypes and their responsiveness to seed mass per plant, the treatments UNI43 and UNI08 not showed significant difference but both are different from BR1 access (Figure 5).

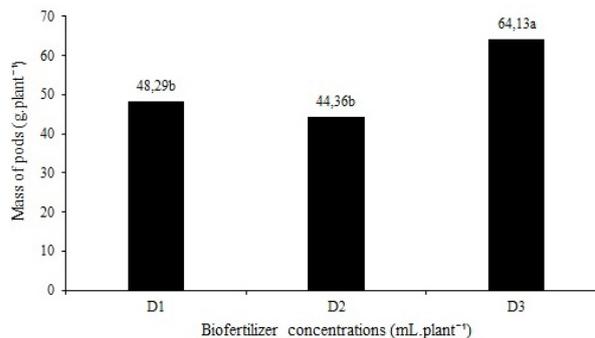
For Santos et al. (2013) equilibrium in production is genetically inherited, so that there is variability within the selection for semiarid environments. Such diversity is mainly found in unimproved peanut subspecies, such as accessions UNI43 and UNI08.

On the other hand, the analysis of the behavior of the MSP for available biofertilizer concentration demonstrated that the D3 treatment induced the best responses for the seed mass gain (Figure 6). Such responses can be explained by fact that the absorption of nutrients by peanuts especially occurs through roots, gynophores and developing fruits.

According to Neto et al. (2012) 80% of the nitrogen translocated to peanut grains is absorbed in the final stages of plant development. This



**Figure 3.** Comparison of the average mass of pods produced by peanut genotypes, Redenção, Ceará, 2018. Equal letters do not differ statistically by the F test at 1% significance.



**Figure 4.** Biofertilizer doses affecting pod mass per peanut plant, Redenção, Ceará, 2018. D1 – dose one; D2 – dose two; D3 – dose three. Equal letters do not differ statistically by the F test at 1% significance.

shows the efficacy of the effects of the highest dose applied in this study for the trait.

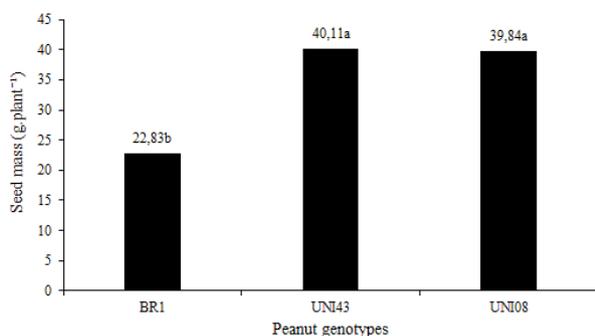
In their study on the production of castor bean seeds fertilized with enriched bovine biofertilizer, Campos et al. (2009) observed the increased number of seeds when the dosage of bovine biofertilizer applied to the soil increased from 600 to 800mL.

Fonsêca (2005) analyzed the increased dry matter and the production of peanut pods following the application of organic fertilizers (cattle tanned manure, poultry tanned manure and Bokash) and mineral fertilization and found that organic fertilizers do not positively affect the seed mass of the species.

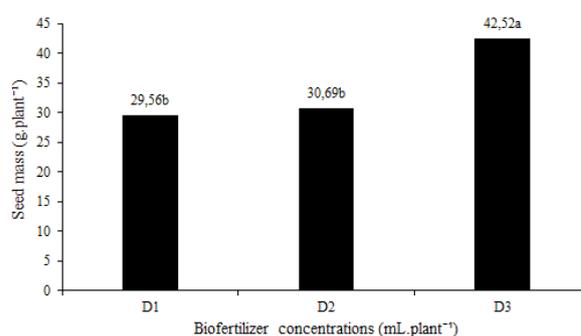
Regarding the length of the root system of the studied genotypes, it can be verified that

the genotype UNI08 presents greater capacity to expand its roots when compared to the other materials (Figure 7). This effect was also affected by biofertilizer doses. D2 and D3 presented the same effect on root system increase (Figure 8).

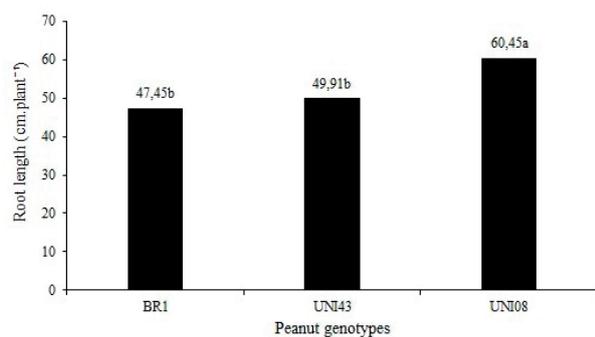
Beltrão Júnior et al. (2012) investigated the yield of cowpea fertilized with different doses of organic biofertilizer, at a dosage of 3L, and found increased root length. In the vegetative phase, the plants spend a great amount of energy for their fixation in the soil, mainly with exploration and emission in the soil. Thus, they become the preferred drainage alternative of photoassimilates, the largest producer of dry matter and area of multiplication of rhizobia.



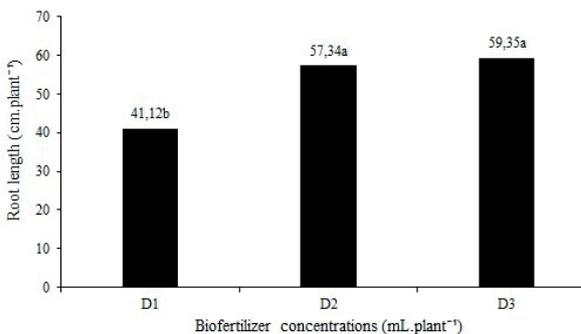
**Figure 5.** Comparison of seed mass averages produced by peanut genotypes, Redenção, Ceará, 2018. Equal letters do not differ statistically by the F test at 1% significance.



**Figure 6.** Biofertilizer doses affecting seed mass per peanut plant, Redenção, Ceará, 2018. D1 – dose one; D2 – dose two; D3 – dose three. Equal letters do not differ statistically, by the F test at 1% significance.



**Figure 7.** Comparison of root mean lengths of peanut genotypes, Redenção, Ceará, 2018. Equal letters do not differ statistically, by the F test tat 1% significance.



**Figure 8.** Doses of biofertilizers affecting peanut root length, Redenção, Ceará, 2018. D1 – dose one; D2 – dose two; D3 – dose three. Equal letters do not differ statistically by the F test at 1% significance.

## CONCLUSIONS

The production and nodulation of peanuts is affected by fertilization via bovine biofertilizer. The 1000 mL dose of biofertilizer was the best treatment for the traits analyzed. The responsiveness of the accessions is genotype-specific and UNI08 stands out from the others.

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#### ANA KELLY S. JULIÃO<sup>1</sup>

<https://orcid.org/0000-0001-6486-6880>

#### LUCAS N. DA LUZ<sup>1</sup>

<https://orcid.org/0000-0002-1060-8963>

#### MARYSSOL T. GADELHA<sup>1</sup>

<https://orcid.org/0000-0002-4629-6172>

#### MATHEUS L. OLIVEIRA<sup>1</sup>

<https://orcid.org/0000-0001-7336-8939>

#### MARIA V.S. SILVEIRA<sup>1</sup>

<https://orcid.org/0000-0003-1573-8692>

#### SAMUEL F.A.O. CASTRO<sup>1</sup>

<https://orcid.org/0000-0003-4151-5732>

#### LENIN P. BARROS<sup>2</sup>

<https://orcid.org/0000-0003-3759-6867>

<sup>1</sup>Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Rua José Franco de Oliveira, s/n, Campus Auroras, 62790-000 Redenção, CE, Brazil

<sup>2</sup>Universidade Federal de Viçosa (UFV), Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900 Viçosa, MG, Brazil

Correspondente to: **Lucas Nunes da Luz**

E-mail: [lucasluz@unilab.edu.br](mailto:lucasluz@unilab.edu.br)

### Author contributions

ANA KELLY S. JULIÃO: Primary developer of the work. Scientific initiation scholarship holder whose project and experiment originated this article. LUCAS N. DA LUZ: Professor advisor and leader of the research group. Author of the original idea of the paper. MARYSSOL T. GADELHA: Scholarship holder of scientific initiation “voluntary” whose project and experiment originated this article. Member of the research group. MATHEUS L. OLIVEIRA: Member of the research group. Field design assembly and evaluation team. MARIA V.S. SILVEIRA: Scholarship holder of scientific initiation “voluntary” whose project and experiment originated this article. Member of the research group. SAMUEL F.A.O. CASTRO: Member of the research group. Field design assembly and evaluation team. LENIN P. BARROS: Student egress from the research group, whose task was to review the final text, adapt to the format of the journal and translation into English language.

