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Development and production of cowpea inoculated with *Bradyrhizobium* strains in a traditional cultivation system in Southwestern Amazonia

Thiago Araújo dos Santos[a,*], Vagner Oliveira Dias[a], Leandro Roberto da Cruz[b], Alcimone Maria da Costa Silva[c], Marcia Reed Rodrigues Coelho[d], Antônia Jerlene Martins de Lima[a] & Eduardo Pacca Luna Mattar[e]

[a]Multidisciplinary Center, Universidade Federal do Acre. Estrada do Canela Fina, Km 12. Cruzeiro do Sul, Acre, Brazil

[b]Instituto Federal de Santa Catarina. Avenida Expedicionários. Canoinhas, Santa Catarina, Brazil

[c]Instituto Federal do Acre. BR-364, Tarauacá, Acre, Brazil

[d]Embrapa Agrobiologia. BR-465, Km 7, Seropédica, Rio de Janeiro, Brazil

[e]Center for Biological and Natural Sciences, Postgraduate Program in Forestry Sciences, Universidade Federal do Acre. BR 364, km 04, Rio Branco, Acre, Brazil

[*]E-mail: thiagosantosac96@outlook.com

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Traditional cowpea cultivars are conserved in the Juruá Valley (Acre) by farmers who adopt a "beach" production system, in lowland areas on the banks of the Juruá River and tributaries. Cowpea inoculation is a sustainable and low-cost technique that has provided an increase in the growth and productivity of the crop, but that has not yet been tested in this production system. The objective of this work was to evaluate the development and production of cowpea inoculated with *Bradyrhizobium* strains in a traditional cultivation system in Southwestern Amazonia. A randomized block design with three replications and four treatments was used: seeds inoculated with strains BR 1808, BR 3262, and BR 3267, and a control without inoculation. Inoculation provided an increase in the majority of the studied variables. The productivity obtained in the treatments with BR 3262 and BR 1808 was superior to the other treatments. The strain BR 3262 showed higher levels of total chlorophyll and nitrogen. Inoculation with *Bradyrhizobium* proved to be a promising technology for the cultivation of cowpea beans in lowland soils of the Juruá River.

Keywords: Biological nitrogen fixation, Fixation, Inoculation, Organic farming

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Cowpea [*Vigna unguiculata* (L.) Walp.], belonging to the Fabaceae family, is a domesticated legume in Africa, with prominent secondary domestication in India[1], being adapted to tropical and subtropical climate regions[2]. The world production of cowpea is 8,986,191 tons, with emphasis on Nigeria, Niger, and Burkina Faso, which are responsible for 77.8% of this production[3]. Cowpea is an essential crop for food security[4].

Like other legumes, the cow pea has the ability to associate with several strains of rhizobia, which provide plants with the nitrogen necessary for their development, through a process called biological nitrogen fixation (BNF)[5]. BNF has proven to be indispensable for the sustainability of agriculture, given the increase in productivity through the supply of nitrogen to crops, with low economic cost and reduced environmental impact[6,7].

*Corresponding author

Brazil is a reference in the production of inoculants containing strains of nitrogen-fixing bacteria, with emphasis on the works of Johanna Döbereiner (*in memoriam*). According to the Ministry of Agriculture, Livestock and Supply (MAPA), four strains are recommended for the production of commercial inoculants for the cowpea in Brazil[8]. It is worth mentioning that the efficiency of these bacteria can be influenced by the edaphoclimatic conditions of the region and the cultivar used[9,10].

Inoculation with *Bradyrhizobium* in cowpea has led to an increase in several production components of the culture and in nodulation, thus guaranteeing its consistent use in cultivation environments[11-14].

Experiments with cv. “BRS Guariba” in the Brazilian cerrado, evaluating the efficiency of inoculation of rhizobia in two municipalities, showed that the strain BR 3262 presented increases of 67.5% and 23.5% in the productivity of the culture in relation to the cultivation without iconulation[15]. On the other hand, another study evaluating the influence of inoculation of rhizobia on the production of cowpea, showed lower production with strains BR3262 and BR3267 when compared to the control without inoculation, although the native soil microorganisms can also be efficient in increasing crop yield[16].

Acre, in the Brazilian Amazon, stands out as an important *on farm* conservation center for traditional cultivars of the species[17,18]. Cowpeas arrived in Brazil initially through the Northeast region, during colonization and the trafficking of black people for slave labor[19]. It is believed that the traditional cultivars produced in Acre were brought by northeastern migrants during the Amazon rubber boom[20].

In this state, the "beach production system" is used; in which traditional farmers cultivate this species, during the low season, in areas neighboring the muddy rivers that come from springs in the Andes (Fig. 1). A study carried out in Southwestern Amazonia, showed that bacteria present in this cultivation system can be associated with the roots of cowpea plants[21].

This cultivation system is carried out by traditional farmers and presents the potential to be certified as organic[22]. The system is also implemented in other micro-regions, river basins, and states in the Amazon[23,24]. Despite its importance for the conservation of genetic resources and for food sovereignty of traditional populations, there are no studies evaluating the use of inoculants in this production system, which would be a low-cost technology, consistent with an organic production system.

In this context, the objective of the current work was to evaluate the development and production of cowpea inoculated with *Bradyrhizobium* strains in a traditional cultivation system in Southwestern Amazonia.

Fig. 1 — Areas with traditional *V. unguiculata* system in properties adjacent to the Juruá River, Acre, Brazil

Material and Methods

Characterization of the environment and experimental design

The work was conducted on private property in the Praia Grande community (7°40'02.2 "S 72°40'05.9"W) located on the banks of the Juruá River in the municipality of Cruzeiro do Sul, Acre (Fig. 2).

Crutzeiro do Sul belongs to the Brazilian humid tropics, Amazon biome. In this region there are two seasons: the dry season that extends from June to August, and the rainy season that extends from October to May, with an average annual rainfall of 2227 mm, and an average annual temperature of 25°C[25]. Data on precipitation, average, maximum, and minimum temperature in the year of the experiment can be seen in Figure 3.

The experiment was implemented in a randomized block design with 4 treatments and 3 repetitions in a lowland area of the Juruá River, locally denominated "barranco" and containing Fluvic neossol, commonly used for agriculture during low water. The chemical and physical characteristics of the soil in the experimental area are shown in Table 1.

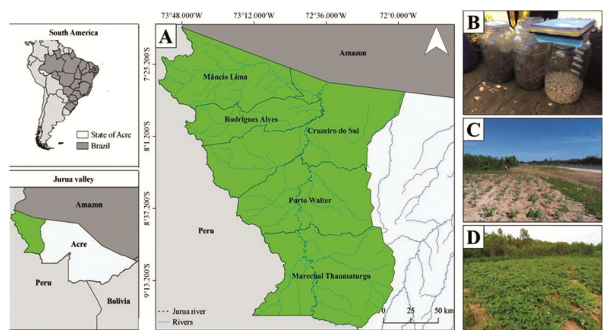

Fig. 2 — Valley of the Juruá River located in the state of Acre (A), inoculated cowpea seeds (B), and detail of the cultivation area (C and D)

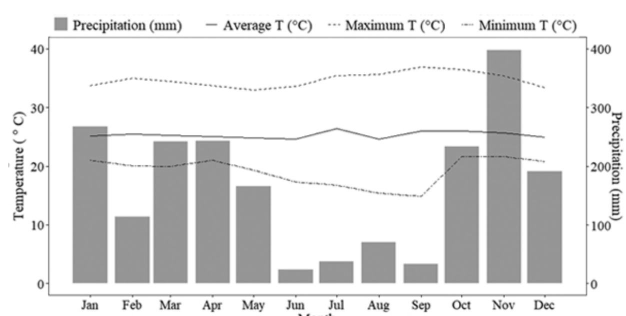

Fig. 3 — Precipitation (mm), maximum (°C), average (°C), and minimum temperature (°C) in the city of Cruzeiro do Sul - AC during the year of the experiment (2018). Source: National Institute of Meteorology – INMET

Table 1 — Chemical and physical characteristics of the soil of the implantation area of the cowpea experiment inoculated with *Bradyrhizobium* strains in the floodplain of the Juruá river, Acre, Brazil.

Soil attribute	Values
Chemical	
pH (H_2O)	6.42
pH ($CaCl_2$)	5.49
P (mg dm^{-3})	75.00
K ($cmol_c$ dm^{-3})	0.35
Ca ($cmol_c$ dm^{-3})	10.17
Ca + Mg ($cmol_c$ dm^{-3})	13.2
Al ($cmol_c$ dm^{-3})	0.00
H + Al ($cmol_c$ dm^{-3})	2.17
Physical	
Sand (g kg^{-1})	555.00
Silt (g kg^{-1})	170.00
Clay (g kg^{-1})	275.00
Additional	
SB ($cmol_c$ dm^{-3})	13.59
T ($cmol_c$ dm^{-3})	15.76
V (%)	86.23

SB – Sum de Bases
T – CTC pH 7
V – Base saturation

Implementation and maintenance of the experiment

Cowpea seeds of the traditional variety "Quarentão" were used, which is grown by farmers in the region in the beach production system[18]. Weed control during the experiment was carried out manually whenever necessary.

The treatments consisted of seeds inoculated with strains of (i.) *Bradyrhizobium sp.* SEMIA 6146 (=BR 1808), (ii.) *Bradyrhizobium pachyrhizi* SEMIA 6464 (=BR 3262), (iii.) *Bradyrhizobium yuanmingense* SEMIA 6462 (=BR 3267), and (iv.) control consisting of seeds without inoculation. The strains were acquired from the cultivar collection of Embrapa Agrobiologia - Seropédica - RJ. The seeds were inoculated 1 hour before planting in the proportion of 500 g of tufted inoculant for each 50 kg of seeds, using a 10% sugary solution for better inoculum adherence, in the recommended concentration of 300 mL for each 50 kg of seed[26].

Sowing was carried out in June 2018 in plots of 16 m^2, with 4 rows spaced at 0.6 m and 0.4 m between plants, with a density of 41,666 plants per hectare. Five seeds were deposited per hole and 20 days after sowing, selective thinning was carried out manually.

Measured variables and statistical analysis

A total of 43 days after sowing (DAS), the following variables were evaluated: plant height (cm) using a graduated ruler, the diameter of the plant base (mm) with the aid of a caliper, and number of branches. At the beginning of flowering (50 DAS), two plants were collected by repetition to determine the number of nodules, plant length (cm) with the aid of a measuring tape, dry weight of the nodules, root, and aerial part (g), and micro (mg Kg^{-1}) and macronutrient content (g Kg^{-1}).

The micro and macronutrient contents were determined by collecting 30 trefoils from each replicate. The samples were washed with distilled water and dried in an oven with forced air circulation at 65°C for 72 h, then ground in a Willey-type mill and placed in paper bags. Aliquots were taken from each crushed sample for subsequent determination of the levels of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S) and magnesium (Mg) and micronutrients boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Nutrient analyses were performed at the Laboratory of Soil and Plant Analysis, Faculty of Agricultural and Veterinary Sciences - UNESP/Botucatu Campus, following the suggested standards for nutrient determination[27].

For root and nodule evaluations, 0.064 m^3 of soil was collected. The samples were taken to the laboratory and with the aid of sieves 2 mm thick and 55 cm in diameter; the nodules were separated from the roots.

The chlorophyll index was determined using the portable electronic equipment ClorofiLOG® 1030 and the readings were performed on 60% of the trefoil leaf blade of the middle third of each plant, avoiding the ribs. The ClorofiLOG® 1030 is capable of performing measurements in three ranges of light length, two in the red (635 nm and 660 nm) and one in the infrared (880 nm). This index, through a color reading, estimates the amount of chlorophyll. Readings were taken 16, 29, 44, 52, and 58 days after sowing the crop.

The dry pods were harvested 83, 90, and 98 days after sowing, and at the end the grain yield was determined. To determine the dry mass, the samples were packed in paper bags and then dried for 72 h at 65°C in a forced air circulation oven.

The data were submitted to analysis of variance by the F test, adopting 5% significance and the averages compared by the Scott Knott test. The total chlorophyll indices were adjusted to the polynomial regression up to the 3rd order. The statistical analysis was performed with the aid of R software operated in RStudio 3.5.1[28], with the aid of the ggplot 2 3.3.2 package[29].

Results and Discussion

The reading of the chlorophyll index in cowpea leaves revealed higher averages for treatments that received inoculation. The strain BR 3262, adjusted to the growing cubic model, presented higher averages of total chlorophyll. The control was adjusted to an increasing linear model, presenting averages similar to the treatments inoculated with strains BR 3267 and BR 1808, with the exception of 43 DAS (Fig. 4).

The SPAD chlorophyll meter serves to indirectly estimate nitrogen (N) status in various species, including cowpea[30]. It should be emphasized that nitrogen is an important component of the molecules of chlorophyll A and chlorophyll B[31]. Since there is a strong correlation between the chlorophyll reading and N accumulation in the cowpea leaf area[32], the results evidenced in this experiment indicate that BNF occurred efficiently in the treatments inoculated with BR 3262, in the majority of the readings performed during the experiment.

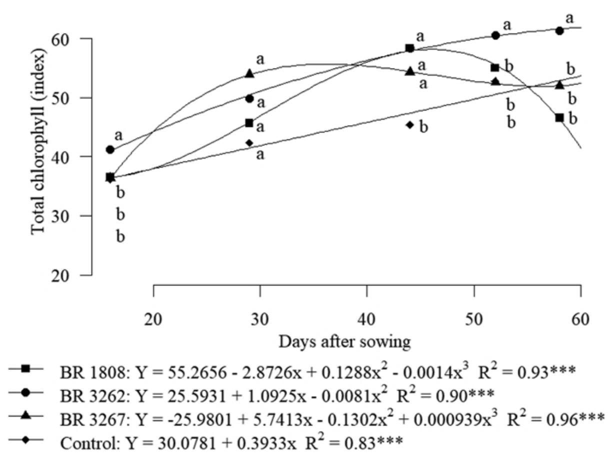

Fig. 4 — Total chlorophyll index of cowpea plants inoculated with Bradyrhizobium strains in the floodplain of the Juruá river, Acre, Brazil. Distinctive lowercase letters between treatments within each evaluation date indicate a difference according to the Scott-Knott test at 5% probability

Inoculation provided a significant increase in the height of cowpea plants (Table 2). The strains of *Bradyrhizobium* provided an average increase of up to 6 cm in plant height when compared to the control. However, a work with cv. BRS Novaera in Southeastern Bahia, reported divergent results from those found in the current work, since the strains BR3262 and BR3267 of *B. elkanni* and *B. japonicum* respectively did not differ from the control[33]. For the variables stem diameter and number of branches, there were no statistical differences between treatments (Table 2).

Inoculation with strains BR 1808 and BR 3262 significantly increased the nodulation of the cowpeas and, concomitantly, the dry mass of the nodules in relation to plants not inoculated and inoculated with BR 3267 (Table 3). These results were also evidenced in another study after inoculation with *Bradyrhizobium*[34]. In studies with the cultivar BRS Guariba in two municipalities of Maranhão, no increase in the number of nodules was observed in the inoculated plants, however there was an increase in the dry weight of the nodules when compared to the control without inoculation and without N[35].

Table 2 — Performance of cowpea inoculated with *Bradyrhizobium* strains in the floodplain of the Juruá river, Acre, Brazil.

Treatment	Plant height (cm)	Stem diameter (cm)	Number of branches (no. plant^{-1})
Control	16.33 b	0.87 a	7.00 a
BR1808	22.33 a	0.97 a	6.33 a
BR3262	20.83 a	0.80 a	7.67 a
BR3267	22.00 a	0.83 a	7.33 a
CV (%)	6.9	8.6	12.7
Average	20.38	0.87	7.08

CV – Coefficient of variation

Averages followed by the same lowercase letter in the column do not differ according to the Scott-Knott test at 5% probability.

Table 3 — Performance of cowpea inoculated with *Bradyrhizobium* strains in the floodplain of the Juruá river, Acre, Brazil.

Treatment	Number of nodules (no. plant$^{-1)}$	Dry nodule weight (g plant^{-1})	Dry root weight (g plant^{-1})	Dry aerial part weight (g plant^{-1})	Total dry weight (g plant^{-1})	Plant length (cm)
Control	146.0 c	0.67 b	3.18 b	80.93 c	84.11 c	153.33 b
BR1808	254.5 a	1.21 a	3.55 b	89.85 b	93.40 b	171.67 a
BR3262	248.5 a	1.47 a	4.17 a	99.06 a	103.23 a	174.33 a
BR3267	172.0 b	0.61 b	3.00 b	71.95 d	74.96 d	157.50 b
CV (%)	3.02	21.2	8.84	3.9	3.67	2.2
Average	205.3	0.98	3.47	84.45	88.92	164.21

CV – Coefficient of variation

Averages followed by the same lowercase letter in the column do not differ according to the Scott-Knott test at 5% probability.

Nodulation depends on soil fertility, with lower nodulation being observed for cowpea in fertilized treatments compared to non-fertilized crops[36].

Regarding root dry mass, there was a significant increase with the use of the BR 3262 strain when compared to the other treatments. On the other hand, a study showed that the BR3262 and BR3267 strains did not differ in relation to root dry mass for the BRS Pujante cultivar[37]. Other studies have also reported the efficiency of inoculation for increasing the dry weight of cowpeas[38,39].

Plant length responded significantly to inoculation with strains BR 3262 and BR 1808 (Table 3). It is worth noting that the influence of inoculation on plant length differs for each cultivar, as shown by Souza *et al.*[10] who tested the inoculation of *B. japonicum* and obtained an increase in length only in IPA 207, and not for Manteiguinha and BRS Tumucumaque.

The increase in dry mass of the aerial part and total due to inoculation with the strain BR 3262 can be attributed to the effectiveness of this strain of *Bradyrhizobium* (Table 3). Similar results were found by Yoseph *et al.*[38] who observed an increase in the dry mass of the aerial part with *Bradyrhizobium* strains. Marinho *et al.*[40] found no differences in the dry mass of the aerial part of cowpea between treatments with strains BR 3267 and BR 3262 and the control.

Other studies carried out in different regions show that according to the cultivar and strains of rhizobia, there are different responses in the production of dry plant mass. Costa *et al.*[41] evaluating the response of cultivars BRS Guariba and BR 17 Gurguéia to inoculation with *Bradyrhizobium* at 45 days, obtained an increase in the dry mass of the aerial part only in the cultivar BRS Guariba.

In a study with cv. Vinagre in the state of Tocantins, Borges *et al.*[42] did not observe an increase in the total dry mass of cowpea plants with strains BR3262 and BR3267. These results differ from those found in the current work, since the inoculation provided an increase in the total dry mass of the plants.

The accumulation of macronutrients is shown in Table 4. The strain BR 3262 provided a significant increase in the levels of N and S. The levels of N obtained, regardless of the treatment, are above the range considered for the culture, which varies between 18 and 22 g kg^{-1} according to Malavolta *et al.*[27]. With respect to the levels of S, the inoculation established averages superior to the optimum range of the culture. These results differ from those found by Moreira *et al.*[39], who did not find raised the levels of N and S after inoculation.

Nascimento *et al.*[37] also observed a significant increase in N content in the aerial part of bean plants with the use of the BR 3262 strain, in relation to the control and BR3267.

Regarding the K content, the inoculation treatments with the strain BR 3267 presented higher averages, however, regardless of the treatment, the levels of this nutrient were below the range considered adequate by Malavolta *et al.*[27]. Moreira *et al.*[39] also showed an increase in K levels with the inoculation of cowpea.

With regard to micronutrient contents, inoculation with BR3262 provided a significant increase in B and Fe contents in relation to the control treatment, with an increase of 36.5% for B and 32.2% for Fe (Table 5). On the other hand, there was a significant increase in Mn content only for strain BR3267, with an increase of 24.8% in relation to the control. Despite the variations found between treatments, only the Cu and Zn levels were above those recommended for the culture, while the others were below the critical level considered by Malavolta *et al.*[27].

Table 4 — Leaf macronutrient content of cowpea inoculated with *Bradyrhizobium* strains in the floodplain of the Juruá river, Acre, Brazil.

Treatment	N	P	K	Ca	Mg	S
	Macronutrients (g kg^{-1})					
Control	44.12 b	2.67 a	15.10 b	24.40 a	3.13 a	1.92 b
BR1808	46.83 b	2.63 a	15.50 b	27.10 a	3.23 a	2.25 b
BR3262	52.78 a	2.60 a	15.67 b	29.13 a	3.20 a	2.71 a
BR3267	45.15 b	2.77 a	17.03 a	23.30 a	3.13 a	2.22 b
CV (%)	5.19	3.94	3.03	8.31	3.02	8.76
Average	47.22	2.67	15.83	25.98	3.18	2.27

CV – Coefficient of variation

Averages followed by the same lowercase letter in the column do not differ according to the Scott-Knott test at 5% probability.

Table 5 — Leaf micronutrient content of cowpea inoculated with *Bradyrhizobium* strains in the floodplain of the Juruá river, Acre, Brazil.

Treatment	B	Cu	Fe	Mn	Zn
	Micronutrients (mg kg^{-1})				
Control	14.88 c	12.33 a	442.67 b	105.00 b	53.33 a
BR1808	17.05 b	13.33 a	456.67 b	103.00 b	57.67 a
BR3262	20.31 a	14.00 a	589.50 a	78.67 c	57.00 a
BR3267	13.49 c	11.33 a	470.50 b	131.00 a	53.33 a
CV (%)	8.44	8.77	10.5	11.62	6.71
Average	16.43	12.75	489.83	104.41	55.33

CV – Coefficient of variation

Averages followed by the same lowercase letter in the column do not differ according to the Scott-Knott test at 5% probability.

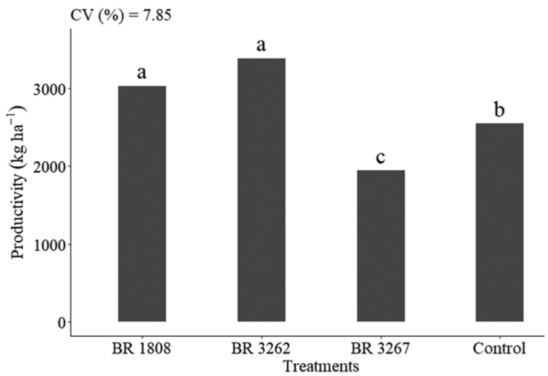

Fig. 5 — Productivity of cowpea inoculated with *Bradyrhizobium* strains in the floodplain of the Juruá river, Acre, Brazil. Averages followed by the same lowercase letter do not differ according to the Scott-Knott test at 5% probability

In relation to productivity, treatments containing the inoculants BR 3262 and BR 1808 presented higher averages with 3384 and 3031 kg ha^{-1} respectively (Fig. 5). The use of these inoculants increased crop productivity, which, according to Silva *et al.*[43] the national average is 366 kg ha^{-1}. In addition to providing an increase in crop yield, strain BR 3262 was also efficient in BNF, different from other treatments. Therefore, the use of inoculants with this strain provided high productivity for the cowpea in the production system adopted.

There was a 38.8% increase in productivity with the use of BR 3262, when compared to the control. Chaves *et al.*[44] in Roraima studying the same strain in an area left fallow for two years, found a 62.5% increase in the productivity of cultivar BRS Guariba with the same strain.

In an upland environment in the Amazon, for the cowpea cultivar BRS Tumucumaque, inoculation with strains UFLA 3-84 and INPA 03-11B (*Bradyrhizobium*) promoted higher grain yield compared to strains BR 3262 and BR 3267[16]. This demonstrates the need for research testing the effects of other inoculants.

The BR 3267 strain showed lower productivity when compared to the other treatments, with an average of 1941 kg ha^{-1}. According to the results found by Marinho *et al.*[9], the efficiency of the inoculation of strains BR3262 and BR3267 is influenced by the environment and the cultivar used.

Despite the variations obtained in cowpea productivity in this experiment, the averages found, independent of the treatment, are higher than the national average for this crop. This indicates that the population of rhizobia native to the floodplain soils of the Juruá River is sufficient to guarantee high yields of cowpea, however that inoculation with strains BR 3262 and BR 1808 can further increase crop productivity. In addition, aiming to achieve greater benefits, the co-inoculation of *Bradyrhizobium* spp. and *Azospirillum brasilense*, resulted in an increase in the production of cowpea[45].

Inoculants are products authorized for use in organic systems, and the technology presented and discussed in this work is consistent with the national policy of agroecology and organic production in Brazil. It does not present a risk of contamination to farmers and river waters, considering that the beach system uses adjacent areas. Despite the promising results, it is worth mentioning that this technology should be tested in other traditional cultivars grown under this production system. In addition, research projects should be considered that aim to obtain native strains from river beaches.

Conclusion

Inoculation with *Bradyrhizobium* strains provided an increase in height, total chlorophyll index, number of nodules, and dry weight of the nodule, root, aerial part and total, micro and macronutrient contents, and productivity of cowpea.

The *Bradyrhizobium* strain BR 3262, in addition to raising the productivity in relation to the control and the strain BR 3267, presented higher averages of total chlorophyll and N levels.

The use of *Bradyrhizobium* strains proved to be an efficient technology for the cultivation of cowpea in floodplain soils of the Juruá River, with an increase in production of up to 32.8% in relation to the control.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

The entire team worked together to complete this article. Authors VOD, AJML and AMCS worked on collecting field data. The manuscript was written and finalized by TAS, LRC, and EPLM. Data analyses were performed by TAS. MRRC provided the rhizobia strains to carry out the work and helped with the initial writing. The final manuscript was read and approved by the entire team.

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