MICROBIAL CROSS-FEEDING AND ITS EFFECTS ON COWPEA (VIGNA UNGUICULATA) PLANT GROWTH PROMOTION

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ABSTRACT

Purpose: To evaluate the in vitro metabolic compartmentalization between strains of actinobacteria and rhizobia isolated from Brazilian semiarid soils and observe the effect of their coinoculation on the development of cowpea plants.

Theoretical Framework: The study is based on the importance of microbial interactions in soil, such as metabolite and enzyme sharing, for nutrient cycling and plant growth promotion.

Method: Twenty-six rhizobial strains and 23 actinobacteria were cocultured in vitro in media containing xylan, pectin and calcium phosphate. Strains were characterized regarding compatibility on different substrates. Two Streptomyces sp. strains and eight Bradyrhizobium sp. strains were selected and coinoculated in cowpea plants in a greenhouse to evaluate plant development and gas exchange.

Results: Compatibility between strains varied significantly among the tested substrates, being higher in pectin and phosphate. Coinoculation resulted in increased values of several biometric parameters compared to standard treatments with only rhizobia or nitrogen fertilization.

Conclusions: The association of microorganisms with distinct metabolic capabilities, such as enzyme production, promotes bacterial coexistence and facilitates plant development through complementarity mechanisms.

Originality: There are scarce studies on metabolic interactions and growth promotion involving actinobacteria and rhizobia from semiarid soils.

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Keywords: Microbial Interactions, Resource Sharing, Growth Promotion, Cowpea, Semi-arid.

ALIMENTAÇÃO CRUZADA MICROBIANA E SEUS EFEITOS NA PROMOÇÃO DO CRESCIMENTO VEGETAL DO FEIJÃO-FRADE (VIGNA UNGUICULATA)

RESUMO

Objetivo: Avaliar a compartimentalização metabólica in vitro entre linhagens de actinobactérias e rizóbias isoladas de solos semiáridos brasileiros e observar o efeito da sua coinoculação no desenvolvimento de plantas de feijão-fradinho.

Estrutura teórica: O estudo baseia-se na importância das interações microbianas no solo, como a partilha de metabolitos e enzimas, para o ciclo de nutrientes e a promoção do crescimento das plantas.

Método: Foram cultivadas in vitro 26 estirpes rizobiais e 23 actinobactérias em meios contendo xilana, pectina e fosfato de cálcio. Foram caracterizadas estirpes quanto à compatibilidade e diferentes substratos. Duas linhagens de Streptomyces sp. e oito linhagens de Bradyrhizobium sp. foram selecionadas e coinoculadas em plantas de feijão-fradinho em estufa para avaliar o desenvolvimento da planta e a troca de gases.

Resultados: A compatibilidade entre estirpes variou significativamente entre os substratos testados, sendo maior em pectina e fosfato. A coinoculação resultou em valores aumentados de vários parâmetros biométricos em comparação com tratamentos padrão com apenas rizóbia ou fertilização nitrogenada.

Conclusões: A associação de microrganismos com capacidades metabólicas distintas, como a produção de enzimas, promove a coexistência bacteriana e facilita o desenvolvimento da planta através de mecanismos de complementaridade.

Originalidade: Existem estudos escassos sobre interações metabólicas e promoção do crescimento envolvendo actinobactérias e rizóbias de solos semiáridos.

Palavras-chave: Interações Microbianas, Compartilhamento de Recursos, Promoção do Crescimento, Feijão-Fradinho, Semiárido.

INTRODUCTION

Caatinga is an endemic biome of the Brazilian semiarid region that occupies a large area in the northeast of the country. The presence of a hot and dry climate, with low and concentrated rainfall periods, combined with intense sunlight and high temperatures, makes this environment restrictive for some species (Silva et al., 2020). These conditions can be unfavorable for the development of microorganisms in the soil, reducing not only their abundance, but also their diversity, resulting in significant variations in soil microbial functions and decreasing their stability (Gorlach-Lira; Coutinho, 2007; Araújo et al., 2013; Maestre et al., 2015).

Nutritional resources in the soil come mainly from plant biomass. In this nutritional scenario, several poorly soluble and complex structured compounds, such as cellulose, pectin, hemicelluloses and lignin, make up the main sources of C and energy (Harindinntwali; Zhou; Yu, 2020). The presence of microbial extracellular enzymes capable of decomposing these compounds is essential to maintain the chain of interactions that drives nutrient flow.

Microbial metabolic activity is responsible for the degradation, mineralization of compounds and cycling of nutrients in terrestrial ecosystems (Baldrian, 2017). Whether positive
or negative, these relationships are based primarily on resource availability (Pascual-García; Bonhoeffer; Bell, 2020).

The sharing or exchange of metabolic resources between microorganisms has recently been reported as quite common (Zelezniak et al., 2015; Germerodt et al., 2016; Hoek et al., 2016; Campbell et al., 2018; Pascual-García; Bonhoeffer; Bell, 2020). In this perspective, cooperative behaviors stand out in the dynamics of interaction and organization of microbiomes (Amor; Bello, 2017).

Cross-feeding metabolic interactions can be uni or bidirectional and are driven by complex interactions that result in adaptive advantages (fitness) (D’Souza et al., 2018). According to Douglas (2020), metabolic cooperation as a coinoculation mechanism promotes biomass production and increases the taxonomic and functional diversity of microbial communities. Embree et al. (2015) emphasize that positive relationships between microorganisms, such as cooperation, favor species heterogeneity and help understand the diversity and evolution of different communities in their environments.

Studies try to assess in experimental laboratory and field situations how the interaction between different microorganisms can benefit the participants, and the effects of these associations on other organisms (Freilich et al., 2011; Silva et al., 2019; Gorter; Manhart; Ackermann, 2020). For this, the use of microbial consortia has emerged as a common research tool, helping to design and build equivalent or approximate situations of natural environments, thus allowing the investigation of the dynamics of biological communities (Kong et al., 2018).

Research between microbial groups reveals that combined action is able to provide benefits for promoting plant growth (Sahur et al., 2018; Ju et al., 2019). Among these groups, actinobacteria and rhizobia are recognized for their ability to improve the development of leguminous plant species when in association (Santos et al., 2018).

Actinobacteria are Gram positive, filamentous bacteria with widely known metabolic potential, being found in the most diverse environments (Olanrewaju; Babalola, 2019). Rhizobia represent a group of Gram-negative bacteria with the aptitude to associate with roots and stems of legumes, being responsible for biological nitrogen fixation (Dicenzo et al., 2019).

Both groups are present in semiarid environments and have important ecological functions (Pinheiro et al., 2014; Alves et al., 2016).

Interspecific interactions play a fundamental role in the dynamics of microbial communities (Gorter; Manhart; Ackermann, 2020), however there are numerous gaps in understanding how these interactions, especially cooperation at the bacterial level, can be responsible for the coexistence of microorganisms in natural environments, and how these relationships influence the dynamics of the ecosystem at the macro level. Thus, based on the hypothesis that there is positive metabolic interaction between actinobacteria and rhizobia from the same semiarid region, the objective was to evaluate in vitro the presence of metabolic interactions between actinobacteria and rhizobia and their influence in vivo on the plant development of cowpea (Vigna unguiculata) plants.

2 METHODS

2.1 Actinobacteria and Rhizobia

Twenty-six rhizobial strains and 16 actinobacteria were used, isolated from rhizosphere samples of 12 legumes collected during the dry season from August 20 to 24, 2012. The rhizobial strains were authenticated and characterized by Silva et al. (2014) and Feitoza et al. (2015) as Bradyrhizobium spp (L84, L85, L86, L87, L89, L90, L92, L93, L94, L95, L97, L99, L101, L102, L103, L104, L107, L108, L109) and Burkholderia spp (L91, L96, L105). Strains L83, L98, L100 and L110 were not identified. The actinobacterial strains were characterized...
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2.2 Selection of Strains for the in Vitro Metabolic Interaction Test

Actinobacterial strains that presented Enzymatic Index (EI) ≥1.5 on xylan and pectin substrates and Solubilization Index (SI) ≥1.5 on the inorganic substrate calcium triphosphate were selected for the in vitro metabolic interaction test with rhizobial strains that, in the same tests to which the actinobacteria were subjected, did not show metabolic activity on one of the aforementioned substrates.

2.3 In Vitro Metabolic Interaction Test

In the experimental design, each rhizobial strain deficient in degrading a given substrate was cultured in consortium with an actinobacterial strain capable of decomposing that compound. The actinobacterial strains were inoculated on culture media with specific substrates (xylan, pectin and calcium phosphate) in the form of spots in quadruplicate. The plates were incubated at 28 ± 2°C for 10 days. After this period, one milliliter of culture of each rhizobial strain previously grown in YM medium (yeast-mannitol) at 28°C for seven days was transferred to 1.5 mL microtubes. The material was centrifuged (12,000 g for 10 minutes) and the sediment resuspended by vortexing in 100 μL of sterile distilled water. This procedure was repeated twice and then 3 μL of each purified rhizobial culture was inoculated around each actinobacterial colony at a distance of 0.5 to 2.0 cm. The inoculum distribution was performed in duplicate and the multiplication of rhizobial strains in regions closer to the colony was the parameter to diagnose the occurrence of positive metabolic interaction (Cuesta et al., 2012). The test was performed in duplicate with two repetitions.

2.4 Selection of Strains for in Vivo Facilitation Test

The pairs of actinobacteria and rhizobia that presented the highest compatibility values (coinoculation) in the in vitro interaction tests were selected for the in vivo coinoculation test and verification of plant growth promotion characteristics. For selection, actinobacteria that presented promising results in the metabolism of substrates (starch, cellulose, xylan, pectin and calcium phosphate) under different abiotic conditions (pH, NaCl and temperature) were also analyzed.

2.5 Effect of Actinobacterial and Rhizobial Facilitation on Bean Plant Growth

The effect of coinoculation between actinobacteria and rhizobia was evaluated in an experiment carried out in a greenhouse. Cowpea seeds (Vigna unguiculata) were disinfested by immersion in 96° GL alcohol solutions for 30 seconds and then immersed for two minutes in 10% sodium hypochlorite, followed by six consecutive washes with sterile distilled water. Subsequently, they were sown in pots containing vermiculite and sand, in a 2:1 ratio, sterilized in an autoclave for two hours at 121°C and 1.5 atm. The actinobacteria were initially cultured in liquid CD medium (Casein-Dextrose) and incubated for seven days at room temperature under constant orbital agitation of 150 rpm. For treatments with actinobacteria, 3 mL of the...
suspension containing the specific strains were added superficially to each pot on the planting day and after 14 days (Pereira; Neves; Drozdowicz, 1999).

The selected rhizobial strains were individually re-streaked in liquid YM medium, and shaken on an orbital shaker at 150 rpm for seven days. They were then inoculated in the sowing at a ratio of 3 mL/seed at 7 and 21 days after sowing. The trial was completely randomized with four treatments: control (plant); T1 (plant + standard strain BR3301 (Bradyrhizobium sp)); T2 (plant + nitrogen (80 kg/ha)), T3 (plant + actinobacteria1(UB05 (Streptomyces sp) + rhizobia) and T4 (plant + actinobacteria 2 (UB11 (Streptomyces sp) + rhizobia), with four replicates. The trial was conducted in a greenhouse for 30 days and the soil moisture was kept at field capacity.

2.5.1 Evaluation of foliar gas exchange

Foliar gas exchanges were evaluated during the vegetative phase, at 28 days, in the morning (8 am to 10 am), using an infrared gas analyzer (IRGA (LCi ADC BioScientific model, England)), in an open system under saturating light and environmental conditions. The SPAD index was determined using a portable SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd). The variables analyzed were: ambient CO2 (Cref), internal CO2 concentration (Ci), photosynthesis (A), stomatal conductance (gs), transpiration rate (E), ratio between internal and external CO2 concentration (Ci/Ca) and carboxylation efficiency (A/Ci).

2.5.2 Evaluation of vegetative development

After 30 days, the plants were collected and the material was placed in a forced air circulation oven at 65°C for approximately 72 hours until reaching constant mass. Subsequently, the material was weighed on an analytical balance. The vegetative development attributes evaluated were: number of nodules per plant (NN), dry mass of nodules (MSN), root dry mass (MSR), shoot dry mass (MSA), leaf dry mass (MSF), plant height (PH), number of leaves (NL), root size (RS) and leaf area (LA). The relative efficiency (RE) of inoculation was calculated by the ratio between MSA and MSA of treatment 2 (plant + 80 kg/ha N nitrogen), multiplied by 100 (Bergersen et al., 1971).

2.6 Statistical Analysis

The compatibility of metabolic interactions between actinobacteria and rhizobia was analyzed through the construction of an interaction network from binary data (0 for incompatibility and 1 for compatibility). The percentage of compatibility for each actinobacteria-rhizobium pair was calculated by the ratio between the number of compatible pairs and the total possible interactions between pairs, multiplied by 100. The mean compatibility for each group and substrate was evaluated by analysis of variance (ANOVA) with p<0.05. Binomial test was used to assess the probability of positive and negative interactions between microbial pairs in in vitro metabolic interactions. The data collected in the in vivo experiment were subjected to analysis of variance with a significance level of 5% using the F test, and the treatment means were compared using the Tukey test at 5% probability. Pearson's correlation coefficient was calculated to measure the degree of relationship between vegetative development and foliar gas exchange variables. Statistical analyzes were performed using SPSS 20.0 (SPSS Inc, Chicago, USA).
3 RESULTS AND DISCUSSION

3.1 In Vitro Metabolic Interaction Test

The actinobacterial and rhizobial strains showed compatibility of 55.57%, 100% and 90.45% on pectin, xylan and calcium phosphate substrates, respectively. Heterogeneity (Levene p<0.05) and significant differences (F (72,2) = 33.92, p<0.05) were observed in compatibility between substrates (Figure 1).

![Figure 1: Compatibility of interactions on different substrates of actinobacterial and rhizobial strains from the Ubajara National Park - CE.](image)

Note: Means with the same letters do not differ by Tukey's test (p<0.05).

**Source:** Elaborated by the authors (2020).

On the pectin substrate, only the UB16 strain (*Nocardia* sp) showed no compatibility with any of the 11 rhizobial strains (L84, L85, L86, L87, L90, L93, L99, L101, L103, L104 and L110) evaluated (Figure 13). Meanwhile, the *Streptomyces* sp (UB05, UB08, UB11, UB15) and *Terrabacter* sp (UB23) strains were compatible with over 70% of the tested rhizobia (Figure 13). Among the evaluated rhizobia, the *Bradyrhizobium* sp strains (L85, L86, L93, L103 and L104) showed compatibility with over 70% of the analyzed actinobacteria.

The interactions between the actinobacterium UB03 (*Micromonospora* sp) and the 26 rhizobial strains (L83, L84, L85, L86, L87, L89, L90, L91, L92, L93, L94, L95, L96, L97, L98, L99, L100, L101, L102, L103, L104, L105, L107, L109, L110) on the xylan substrate were all positive (Figure 13), showing 100% compatibility.

For the substrate containing calcium phosphate, the UB26 strain (*Streptomyces* sp) showed positive interaction with 90% (L83, L84, L85, L86, L87, L89, L90, L91, L92, L93, L94, L95, L97, L98, L99, L100, L103, L104, L107, L108, L110) of the evaluated rhizobial strains, showing no compatibility only with the *Bradyrhizobium* sp strains (L90 and L101) (Figure 2).

The L85 strain (*Bradyrhizobium* sp) showed compatibility with 90% of the actinobacteria on the pectin substrate and 100% compatibility with the UB03 (*Micromonospora* sp).
sp) and UB26 (Streptomyces sp) strains, indicating the ability to interact with microorganisms on various substrates, such as xylan and calcium phosphate, respectively.

Of the 165 interactions tested between actinobacteria and rhizobia for the pectin substrate, the binomial test revealed no significant differences (p = 0.161) between the 92 (56%) positive interactions and 73 (44%) negative interactions observed, however it was possible to identify that of the 15 actinobacterial strains, six (UB05, UB08, UB11, UB15, UB23, UB24) exhibited almost 100% compatibility proportions (Figure 3), demonstrating greater chances of interspecific interactions.

Figure 2: Metabolic interactions on xylan, calcium phosphate and pectin substrates between actinobacteria and rhizobia from the Ubajara National Park - CE.
Source: Elaborated by the authors (2020).

Figure 3: Proportion of positive metabolic interactions on pectin substrate of 15 actinobacterial strains from the Ubajara National Park - CE.
Source: Elaborated by the authors (2020).

It was possible to identify differences in the mean compatibility on the three analyzed substrates between the microbial groups, ranging from 88.37% for rhizobia and 59.35% for actinobacteria, with a coefficient of variation of 23% (Figure 15). The averages of the percentage of compatible strains showed significant differences with F (73.1) = 16.984 and
p<0.05 between the bacterial groups. The results indicated that the rhizobial strains showed more evidence of positive interactions. Therefore, representing greater chances of establishing metabolic exchanges with other microorganisms in a natural environment, which can also result in greater possibilities of positive influence on the nodulation mechanisms between rhizobia and legumes.

Figure 4: Comparison of the mean percentage of compatibility of actinobacterial and rhizobial strains from the Ubajara National Park - CE.
Source: Elaborated by the authors (2020).

3.2 Selection of Strains for in Vivo Coinoculation Test

Two actinobacterial strains, classified at the genus level as Streptomyces sp (UB05 and UB11) and eight rhizobia, of which seven were classified at the genus level as Bradyrhizobium sp (L84, L86, L93, L98, L102, L103, L108) and one strain L110, without classification (Silva et al., 2014; Brito et al., 2015; Feitosa et al., 2015) were selected for the in vivo coinoculation test. The actinobacterial strains were selected for the following characteristics: ability to degrade various substrates under different abiotic conditions (pH, NaCl and temperature) and compatibility with various microorganisms in in vitro metabolic interaction tests. The rhizobia were selected based on the following characteristics: deficiency in degrading different substrates and ability to metabolically interact with other microorganisms in in vitro tests.

3.3 Effect of Facilitation Between Actinobacteria and Rhizobia on Bean Plant Growth

Multivariate analysis of the data collected for the vegetative development of bean plants inoculated with actinobacteria and rhizobia showed significant variation (p<0.05) in some variables submitted to different treatments. Of the 10 variables related to vegetative development analyzed, seven (MSA, RS, MSR, MSF, MSN, LA, RE) showed significant differences (p<0.05) between the treatments submitted, as shown in table 1.

Despite the statistical difference observed in the MSA variable, only the L86UB05 treatment (Bradyrhizobium sp and Streptomyces sp) approached the average of treatments with
the reference strain BR3301 (*Bradyrhizobium* sp) and nitrogen. As for RS, all treatments with actinobacteria and rhizobia showed higher averages than those nitrogenated or with only one inoculated rhizobium (BR 3301). Regarding MSR, seven of the 16 coinoculated treatments presented higher values than the comparatives. A similar pattern was observed in the NN and MSN variables, and the L86UB05 treatment showed an increase of approximately 74% in the number of nodules when compared to the reference (BR3301), demonstrating the promising nature of these microorganisms in promoting plant growth.

Regarding MSF, only the L86UB05 treatment presented an average value close to those observed in the nitrogen treatments and with the reference strain. In the LA variable, only the coinoculation L98UB11 (*Bradyrhizobium* sp and *Streptomyces* sp) presented the highest RE (53.83%), and the treatments L98UB05 (*Bradyrhizobium* sp and *Streptomyces* sp) and L93UB11 (*Streptomyces* sp) presented higher values than the comparatives. A similar pattern was observed in the nitrogen treatments and with a single rhizobium strain (Table 1). Regarding height up to the petiole, the treatment with the reference strain showed a higher average, however, four coinoculation treatments of actinobacteria and rhizobia (L98UB11, L86UB05, L110UB05, L84UB05), had higher averages than observed in the nitrogen treatment, thus indicating the importance of the combined action of microorganisms in plant development (Table 1).

### Table 1: Effect of coinoculation on shoot dry mass (SDM), root dry mass (RDM), leaf dry mass (LDM), number of nodules (NN), and relative inoculation efficiency (RE).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SDM g plant⁻¹</th>
<th>RDM g plant⁻¹</th>
<th>LDM g plant⁻¹</th>
<th>NN</th>
<th>RE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L102UB05</td>
<td>0.44±0.10b</td>
<td>0.36±0.01ab</td>
<td>0.30±0.06b</td>
<td>6.5±7.18a</td>
<td>30.15±6.91abc</td>
</tr>
<tr>
<td>L102UB11</td>
<td>0.45±0.08b</td>
<td>0.63±0.17ab</td>
<td>0.29±0.06b</td>
<td>4±2.30a</td>
<td>24.45±4.48abc</td>
</tr>
<tr>
<td>L103UB05</td>
<td>0.53±0.07b</td>
<td>0.51±0.14ab</td>
<td>0.36±0.07b</td>
<td>8±3.55a</td>
<td>35.60±4.14ab</td>
</tr>
<tr>
<td>L103UB11</td>
<td>0.54±0.14b</td>
<td>0.67±0.16ab</td>
<td>0.37±0.11b</td>
<td>18.25±16.09a</td>
<td>29.32±7.85abc</td>
</tr>
<tr>
<td>L108UB05</td>
<td>0.44±0.09b</td>
<td>0.32±0.06b</td>
<td>0.28±0.09b</td>
<td>25±10.89</td>
<td>29.98±6.48abc</td>
</tr>
<tr>
<td>L108UB11</td>
<td>0.40±0.25b</td>
<td>0.51±0.11ab</td>
<td>0.28±0.19b</td>
<td>16±3.16</td>
<td>22.02±13.8bc</td>
</tr>
<tr>
<td>L110UB05</td>
<td>0.52±0.25b</td>
<td>0.48±0.20ab</td>
<td>0.37±0.15b</td>
<td>27±17.64a</td>
<td>35.60±17ab</td>
</tr>
<tr>
<td>L110UB11</td>
<td>0.54±0.13b</td>
<td>0.95±0.20a</td>
<td>0.38±0.09b</td>
<td>29.5±7.93a</td>
<td>29.32±7.48abc</td>
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<tr>
<td>L84UB05</td>
<td>0.55±0.05b</td>
<td>0.27±0.05b</td>
<td>0.37±0.05b</td>
<td>18.75±14.68a</td>
<td>37.47±6.59ab</td>
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<td>L84UB11</td>
<td>0.33±0.15b</td>
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<td>17.92±8.3bc</td>
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<td>L86UB05</td>
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<td>41.75±49.27a</td>
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<td>0.46±0.06b</td>
<td>26±2.12a</td>
<td>35.40±4.94ab</td>
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<td>BR 3301</td>
<td>0.79±0.53ab</td>
<td>0.57±0.41ab</td>
<td>0.55±0.37ab</td>
<td>24±16.3a</td>
<td>33.83±36.2a</td>
</tr>
</tbody>
</table>

Note: Means followed by the same letters in the column do not differ by Tukey’s 5% significance test.

CV: Coefficient of variation.

Source: Elaborated by the authors (2020).

The relative inoculation efficiency between treatments showed significant variation (p<0.05) with F(17.54) = 3.88. The treatment with the reference strain BR3301 (*Bradyrhizobium* sp) presented the highest RE (53.83%), and the treatments L86UB05(*Bradyrhizobium* sp and *Streptomyces* sp) and L98UB05(*Bradyrhizobium* sp and *Streptomyces* sp) presented RE of 48.04% and 44.8% respectively, approaching the average observed in BR3301. Regarding vegetative development, the treatments L86UB05 (*Bradyrhizobium* sp and *Streptomyces* sp) and L98UB11 (*Bradyrhizobium* sp and
*Streptomyces* sp.), presented values higher than or equal to treatments with only rhizobium and nitrogen, standing out from the others.

The data related to foliar gas exchange were subjected to multivariate analysis. Only the SPAD variable, related to chlorophyll concentration, showed a significant difference (p<0.05) with F(17.54) = 2.27, the others, although not statistically different, showed variations between treatments (Table 2).

Regarding ambient carbon concentration, there were differences between the nitrogen treatments and the standard strain, and only the L84UB11 treatment (*Bradyrhizobium* sp and *Streptomyces* sp) was slightly below the average recorded. The same condition was observed in the internal CO2 concentration variable (Table 2).

Regarding transpiration, the L103UB11 treatment (*Bradyrhizobium* sp and *Streptomyces* sp) presented a higher average, with a 40% increase compared to the BR3301 treatment. As for photosynthesis, only the treatments with the BR3301 strain and with nitrogen had higher averages, however three treatments with *Bradyrhizobium* sp and *Streptomyces* sp (L103UB11, L108UB11, L98UB11) and one with an unidentified rhizobium strain (L110UB05) showed averages equal to or greater than the nitrogen treatment.

The ratio between external and internal CO2 concentration showed no significant differences (p>0.05) between treatments, yet treatments cooinoculated with actinobacteria and rhizobia revealed higher averages than those recorded in treatments with the standard strain or nitrogen in this variable.

Carboxylation efficiency was higher in the BR3301 treatment (0.08), and only three treatments (L98UB11, L86UB11, L108UB11) of the 16 treatments with actinobacteria and rhizobia showed averages equal to or greater than those verified in the nitrogen treatment (0.05), as shown in table 2.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SPAD</th>
<th>A (mmol m⁻² s⁻¹)</th>
<th>Ci/Ca</th>
<th>A/Ci</th>
</tr>
</thead>
<tbody>
<tr>
<td>L102UB05</td>
<td>21.85±2.18ab</td>
<td>5.77±1.56a</td>
<td>0.68±0.02a</td>
<td>0.02±0.0a</td>
</tr>
<tr>
<td>L102UB11</td>
<td>18.4±2.44b</td>
<td>7.35±1.43a</td>
<td>0.72±0.09a</td>
<td>0.02±0.0a</td>
</tr>
<tr>
<td>L103UB05</td>
<td>21.57±5.19ab</td>
<td>6.57±2.7a</td>
<td>0.74±0.13a</td>
<td>0.02±0.01a</td>
</tr>
<tr>
<td>L103UB11</td>
<td>33.07±12.2ab</td>
<td>11.64±6.31a</td>
<td>0.72±0.04a</td>
<td>0.04±0.02a</td>
</tr>
<tr>
<td>L108UB05</td>
<td>26.5±4.7ab</td>
<td>7.29±1.62a</td>
<td>0.70±0.11a</td>
<td>0.02±0.00a</td>
</tr>
<tr>
<td>L108UB11</td>
<td>31.15±15.5ab</td>
<td>12.50±5.59a</td>
<td>0.65±0.15a</td>
<td>0.05±0.03a</td>
</tr>
<tr>
<td>L110UB05</td>
<td>31.15±10.7ab</td>
<td>11.01±9.4a</td>
<td>0.72±0.1a</td>
<td>0.0±0.034</td>
</tr>
<tr>
<td>L110UB11</td>
<td>26±8.52ab</td>
<td>7.72±1.85a</td>
<td>0.74±0.09a</td>
<td>0.02±0.00a</td>
</tr>
<tr>
<td>L84UB05</td>
<td>20.6±2.37ab</td>
<td>7.07±1.33a</td>
<td>0.69±0.14a</td>
<td>0.02±0.00a</td>
</tr>
<tr>
<td>L84UB11</td>
<td>20.15±7.41b</td>
<td>4.68±3.15a</td>
<td>0.46±0.32a</td>
<td>0.02±0.01a</td>
</tr>
<tr>
<td>L86UB05</td>
<td>28.77±16.5ab</td>
<td>11.85±5.5a</td>
<td>0.65±0.09a</td>
<td>0.05±0.02a</td>
</tr>
<tr>
<td>L86UB11</td>
<td>22.6±7.7ab</td>
<td>8.12±3.81a</td>
<td>0.70±0.08a</td>
<td>0.03±0.01a</td>
</tr>
<tr>
<td>L93UB05</td>
<td>20.1±16.5b</td>
<td>8.16±2.9a</td>
<td>0.68±0.13a</td>
<td>0.03±0.01a</td>
</tr>
<tr>
<td>L93UB11</td>
<td>29.07±7.7ab</td>
<td>8.15±2.85a</td>
<td>0.71±0.07a</td>
<td>0.03±0.01a</td>
</tr>
<tr>
<td>L98UB05</td>
<td>28.32±5.83ab</td>
<td>9.15±2.9a</td>
<td>0.63±0.05a</td>
<td>0.03±0.01a</td>
</tr>
<tr>
<td>L98UB11</td>
<td>43.27±13.5ab</td>
<td>14.02±10.9a</td>
<td>0.67±0.16a</td>
<td>0.06±0.07a</td>
</tr>
<tr>
<td>BR 3301</td>
<td>41.3±27.6ab</td>
<td>17.26±12.1a</td>
<td>0.40±0.27a</td>
<td>0.08±0.05a</td>
</tr>
<tr>
<td>NITROGENÍO</td>
<td>51.15±6.05a</td>
<td>11.73±12.4a</td>
<td>0.45±0.3a</td>
<td>0.05±0.06a</td>
</tr>
</tbody>
</table>

*Source:* Elaborated by the authors (2020)

The variables related to vegetative growth and foliar gas exchange of bean plants were subjected to Pearson’s correlation analysis. All biometric variables showed significant
correlation (p<0.05) with gas exchange variables, with a strong correlation observed between number of leaves and leaf area with chlorophyll content measurements (SPAD) (Table 10).

Relative efficiency showed moderate correlation with vegetative variables, root dry mass, nodule dry mass, number of nodules, number of leaves and leaf area and with gas exchange variables, external CO$_2$ concentration, transpiration and photosynthesis.

The number of nodules showed a significant (p<0.05), positive and moderate correlation with variables related to transpiration, stomatal conductance and photosynthesis, relevant factors in the plant's vegetative development. Root size was the variable with the least correlation with the other variables, showing a weak correlation with relative efficiency and root dry mass.

Plant height up to the petiole showed a strong correlation with chlorophyll content in treatments, and moderate correlation with other gas exchange variables. Leaf area revealed a strong correlation with chlorophyll content and moderate correlation with transpiration, photosynthesis and carboxylation efficiency.

Based on the relationship between the performance of treatments with actinobacteria and rhizobia and the comparative treatments with the standard strain BR3301 and nitrogen, cluster analysis was performed to identify the formation of groups with similar characteristics.

Cluster analysis allowed us to infer the formation of four groups. With the first group concentrating the treatments that most closely approximated the values presented by the BR3301 treatment. The nitrogen treatment was in the second group. The third group was formed by three different treatments (BR 3301, nitrogen and rhizobia + actinobacteria). While the fourth group consists of treatments that presented overall values in the variables lower than those presented by the comparative treatments (BR3301 and nitrogen).

Making up group 1 are treatments L110UB05, L86UB05, L93UB11, L98UB11, L103UB11 and L108UB11, all consisting of rhizobia belonging to the genus *Bradyrhizobium*, with distinct phenotypic characteristics. And of the six actinobacterial and rhizobial treatments in group one, four are made up of the actinobacterial strain UB11 (*Streptomyces* sp).

The treatments in group 1 showed values greater than or close to the BR3301 treatments in the variables root size, root dry mass, leaf dry mass, nodule dry mass, leaf area and SPAD, demonstrating the promising profile of these microorganisms in promoting plant growth, especially under natural soil interaction conditions in semiarid areas to which they are native.

The positive interaction between all rhizobial strains with the actinobacteria *Micromonospora* sp. in xylan is relevant, as enzyme sharing can increase access to resources and fitness for both species (Kallenbach et al., 2019). Such metabolic interaction was also observed by Benito et al. (2017), corroborating the importance of cooperative relationships in the functioning of microbial communities (Embree et al., 2015).

The absence of pectinolytic enzymes in rhizobia can impair nodulation. However, combined action with bacteria producing these enzymes represents an adaptation through biotic interactions (Kallenbach et al., 2019). The genus *Streptomyces* is recognized for its ability to interact with and decompose plant biomass (Olanrewaju; Babalola, 2019; Book et al., 2016), which was evidenced in compatibility with rhizobia in pectin.

Phosphate solubilization by actinobacteria can benefit the development of plant communities by making this limiting resource available (SHADE et al., 2012). The construction of microbial consortia can enhance this metabolic facilitation in complex natural environments (Kumar; Dubey, 2020).

Various microbial combinations promote plant growth (Lu et al., 2017; Mendes et al., 2020). In this study, coinoculation resulted in improvements in biometric parameters, corroborating the potential of the association between actinobacteria and rhizobia (Htwe et al., 2018; Santos et al., 2018). Gas exchanges were positively related to biometric data, evidencing
the effect of inoculation on plant metabolism. The use of consortia is a relevant tool to investigate microbial interactions in ecosystems (Kong et al., 2018).

4 CONCLUSIONS

Rhizobial strains of the genus *Bradyrhizobium* were able to grow in co-culture with actinobacterial strains of the genera *Streptomyces* and *Terrabacter*, demonstrating metabolic compatibility on pectin, xylan and calcium phosphate substrates. The coinoculation of actinobacteria of the genus *Streptomyces* and rhizobia of the genus *Bradyrhizobium* proved to be able to improve the development of biometric attributes and gas exchanges in cowpea plants. It was observed that the microbial association of strains with different metabolic skills allows microbial coexistence and facilitates the development of surrounding communities.

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