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# *Bukholderia* strains promote *Mimosa* spp. growth but not *Macroptilium atropurpureum*[1]

## Estirpes de *Burkholderia* promovem o crescimento de *Mimosa* spp. mas não o de *Macroptilium atropurpureum*

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**ABSTRACT -** The aim of this study was to evaluate the relationship and symbiotic efficiency of 14 strains of *Burkholderia* isolated from rupestrian grasslands, using *M. atropurpureum* and *Mimosa tenuiflora* as trap plants, with the species *M. atropurpureum*, *Mimosa bimucronata* and *M. foliolosa*. For the nodulation and symbiotic efficiency test in *M. atropurpureum*, long-neck bottles containing nutrient solution were used. The experiments with *Mimosa* spp. were carried out in tubes containing vermiculite (160 $cm^3$) and sand (80 $cm^3$) (2:1). The parameters under evaluation were number of nodules, nodules dry matter production, shoots dry matter, roots dry matter, and total dry matter production for all the species analyzed; and plant height, diameter, and the Dickson quality index for *Mimosa* species. Of the 14 tested strains, two nodulated *M. atropurpureum*; however, they were ineffective in promoting plant growth. All the tested strains established symbiosis with *M. bimucronata*, and 12 strains nodulated *M. foliolosa*. Of these, six promoted growth in *M. bimucronata*, and seven presented symbiotic efficiency in *M. foliolosa*. The strains UFLA 01-739, UFLA 01-748 and UFLA 01-751, isolated from *M. tenuiflora*, and UFLA 04-260 and UFLA 04-405, isolated from *M. atropurpureum*, stood out as potential inoculants for the *Mimosa* species evaluated in this study.

**Key words:** Rupestrian grasslands. Nitrogen-fixing bacteria. Symbiosis. *Mimosa bimucronata*. *Mimosa foliolosa*.

**RESUMO -** O objetivo deste trabalho foi avaliar a relação e a eficiência simbiótica de 14 estirpes de *Burkholderia* isoladas de campos rupestres, utilizando *M. atropurpureum* e *Mimosa tenuiflora* como plantas iscas, com as espécies: *M. atropurpureum*, *Mimosa bimucronata* e *M. foliolosa*. Para o teste de nodulação e eficiência simbiótica em *M. atropurpureum* foram utilizadas garrafas do tipo *long neck*, contendo solução nutritiva. Os experimentos com as *Mimosa* spp. foram realizados em tubetes contendo vermiculita (160 $cm^3$) e areia (80 $cm^3$) (2:1). Os parâmetros avaliados foram número de nódulos, produções de massa de matéria seca de nódulos, da parte aérea, da raiz e total para todas as espécies vegetais analisadas, e altura da planta, diâmetro, e o índice de qualidade de Dickson para as espécies de *Mimosa*. Das 14 estirpes avaliadas, duas foram capazes de nodular *M. atropurpureum*. Entretanto, estas foram ineficientes na promoção de crescimento vegetal. Todas as estirpes testadas estabeleceram simbiose com *M. bimucronata* e 12 estirpes nodularam *M. foliolosa*. Destas, seis promoveram o crescimento de *M. bimucronata* e sete apresentaram eficiência simbiótica em *M. foliolosa*. Destacam-se as estirpes UFLA 01-739, UFLA 01-748, UFLA 01-751 isoladas de *M. tenuiflora* e UFLA 04-260 e UFLA 04-405 isoladas de *M. atropurpureum* como potenciais inoculantes para as espécies de *Mimosa* avaliadas.

**Palavras-chave:** Campos rupestres. Bactérias fixadoras de nitrogênio. Simbioses. *Mimosa bimucronata*. *Mimosa foliolosa*.

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## INTRODUCTION

Bacteria of the *Burkholderia* genus occupy a variety of ecological niches, and are exploited for several purposes, such as biological control, bioremediation, and plant growth promotion (COENYE; VANDAMME, 2003). The first *Burkholderia* strains with nodulation capacity were proven in *Macroptilium atropurpureum* (DC), which were isolated from nodules of *Aspalathus carnosa* Bergius and *Machaerium lunatum* (L. f.) Ducke (MOULIN *et al*., 2001).

Since then, it has been proven the ability of *Burkholderia* species to nodulate *M. atropurpureum* (ANGUS *et al*., 2013; ELLIOT *et al*., 2007; LIMA *et al*., 2009), *Cyclopia* spp. (ELLIOT *et al*., 2007), *Vigna unguiculata* (L.) Walp (SOARES *et al*., 2014), *Phaseolus vulgaris* L. (FERREIRA *et al*., 2012; TALBI *et al*., 2010), and *Mimosa* spp. (BARRET; PARKER, 2005; BONTEMPS *et al*., 2010; CHEN *et al*., 2003, 2005, 2006; REIS JÚNIOR *et al*., 2010).

Although it has been proven the nodulation of *M. atropurpureum* and *Mimosa* spp. by *Burkholderia* strains, the symbiotic effectiveness of these relationships is still unknown. The efficiency of these strains has been mainly reported by means of observations by the nodules internal color, and it was verified the presence or absence of leghemoglobin. Moulin *et al.* (2001) and Barrett and Parker (2005) verified that nodules formed in *M. atropurpureum* were ineffective due to the whitish color, and did not observe the presence of leghemoglobin. Chen *et al.* (2005) suggested that the strains of *Burkholderia* Br3407, Br 3454, Br 3461, Br3469 and MAP3-5 were efficient in *Mimosa pudica* L., *M. diplotricha* C. Wright ex Sauvalle, *M. pigra* L. and *M. acutispula* Benth. due to the internal reddish color of the nodule. In addition to nodules internal color, some authors report the efficiency and/or inefficiency of *Burkholderia* strains by means of the evaluation of nitrogenase activity in *Mimosa* spp. nodules. (CHEN *et al*., 2005; ELLIOTT *et al*., 2007; REIS JÚNIOR *et al*., 2010), and few studies have evaluated the development of the plants inoculated with *Burkholderia* strains. It was observed the efficiency of *Burkholderia phymatum* (STM815$^T$) to promote the growth of *Mimosa pudica* (ELLIOTT *et al*., 2007), but the same was not observed when this species was inoculated with *Burkholderia* Hpud10.4(C) (BARRETT; PARKER, 2006). On the other hand, in *M. atropurpureum*, it was verified greater dry matter production and shoot length of plant when it was inoculated with *Burkholderia tuberum* (STM678$^T$). However, this increase was attributed to other processes rather than biological nitrogen fixation, since the observed nodules were ineffective (ANGUS *et al*., 2013).

Considering the economic and ecological importance of *M. atropurpureum* and *Mimosa* spp., as well as the biotechnological potential of *Burkholderia* strains, this study aimed to verify the relationship and symbiotic efficiency of *Burkholderia* strains isolated from nodules of *M. atropurpureum* and *Mimosa tenuiflora* (Willd.) Poir. cultivated in rupestrian grasslands soils in *M. atropurpureum*, *Mimosa bimucronata* (DC.) Kuntze and *Mimosa foliolosa* Benth. subsp. *pachycarpa* (Benth.) Barneby var. *pachycarpa*.

## MATERIAL AND METHODS

The 14 *Burkholderia* sp. strains evaluated regarding their relationship and symbiotic efficiency in *Macropitilium atropurpureum*, *Mimosa bimucronata* and *M. foliolosa* were isolated from nodules of *M. atropurpureum* and *M. tenuiflora* grown in distinct soils of rupestrian grasslands, located in Serra do Cipó, Minas Gerais, Brazil (CARVALHO, 2010). Table 1 shows the cultural characteristics and origin of these strains. The 14 strains were deposited in the GenBank under the accession number KT957898 to KT957912.

Three experiments were carried out in a greenhouse. Each experiment consisted of 17 treatments: 14 treatments corresponded to the inoculation of each *Burkholderia* strain in nutrient solution (HOAGLAND; ARNON, 1950), containing low mineral N concentration (5.25 mg $L^{-1}$); two uninoculated negative controls, one with low mineral N concentration (5.25 mg $L^{-1}$), and another with high mineral N concentration (52.5 mg $L^{-1}$); and one positive control in nutrient solution with low mineral N concentration (5.25 mg $L^{-1}$), inoculated with a nodulating strain, according to the host plant species UFLA 04-212 - *Bradyrhizobium* sp, for *M. atropurpureum* (FLORENTINO *et al*., 2009), BR3460 - *Burkholderia gladioli* for *M. bimucronata* (FARIA *et al*., 1997) and UFLA 01-750 - *Burkholderia* sp. for *M. foliolosa*. The mineral N sources used were $Ca(NO_3)_2.4H_2O$; $KNO_3$ and $NH_4H_2PO_4$.

Each treatment in the experiment with *M. atropurpureum* consisted of three replications. For the experiment with *M. bimucronata* and *M. foliolosa*, each treatment consisted of eight replications.

The experiment with *M. atropurpureum* was carried out from September 17$^{th}$ 2013 to October 28$^{th}$ 2013, and temperature ranged between 16 to 27 °C. Long neck bottles containing nutrient solution (HOAGLAND; ARNON, 1950) were used in the experiment. The bottles were autoclaved for one hour, at 1.5 kg $cm^{-2}$ pressure and 127 °C. *M. atropurpureum* seeds were surface sterilized

using 98% ethyl alcohol (30 seconds), 2% sodium hypochlorite (2 minutes), and then scarified with pure sulfuric acid (20 minutes). After this period, seeds were subjected to successive washes in sterile distilled water. Afterwards, seeds were placed in petri dishes containing sterilized filter paper and moistened cotton, where they remained for 48 hours in a growth chamber, at 28 °C, for the pre-sprouting seed. One plantlet per bottle was used. The 17 strains were grown in liquid culture medium "79" (FRED; WAKSMAN, 1928) for 3 days for *Burkholderia* strains, and for 5 days for UFLA 04-212 (*Bradyrhizobium* sp.), due to differences in the growth time of the strains under shaking (110 rpm), at 28 °C. In each inoculated treatment, plantlets were microbiolyzed with 2 ml inoculum in minimum concentration of $1x10^8$ bacterial cells $mL^{-1}$. At 40 days, plants were collected to determine number of nodules (NN), shoot dry matter production (SDM), root dry matter (RDM), and total dry matter (TDM), and the result was obtained by the sum of SDM and RDM. Data were subjected to analysis of variance, by using the statistical analysis program SISVAR, version 5.3 (FERREIRA, 2011). The effects of the treatments were compared by the Scott-Knott test at 5% probability.

Experiments in *M. bimucronata* and *M. foliolosa* were carried out, respectively, from July 22nd to October 2nd, 2013, and from September 16th to November 25th, 2013. The temperatures ranged from 13 to 26 °C and from 16 to 27 °C, respectively. Plants were cultivated in 240 $cm^3$ polypropylene tubes, containing vermiculite (160 $cm^3$) and sand (80 $cm^3$) (2:1), and irrigated with nutrient solution (HOAGLAND; ARNON, 1950), according to the plants need. *M. bimucronata* and *M. foliolosa* seeds were disinfected using the same procedure applied in *M. atropurpureum* seeds, and went through breaking dormancy process for 10 minutes in pure sulfuric acid. Afterwards, they were subjected to successive washings in sterile distilled water. After pre-sprouting seed in growth chamber (28 °C for 48 hours), one plantlet was transferred to each tube. Plants were harvested after 70 days to determine plant height and diameter, number of nodules (NN), nodules dry matter production (NDM), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM), which was obtained by the sum of SDM and RDM. Dickson quality index (DQI) was obtained by the following equation (1):

$$DQI = [total\ dry\ matter/(HDR + SRR)] \quad (1)$$

In which: SRR is the ratio between shoot dry matter and root dry matter, and HDR is the ratio between shoot height and stem diameter **(**DICKSON; LEAF; HOSNER, 1960).

Data were subjected to analysis of variance

**Table 1 -** Identification and origin of the 14 *Burkholderia* strains used in authentication trials, and symbiotic efficiency in *M. atropurpureum*, *M. bimucronata* and *M. foliolosa*, (CARVALHO, 2010)

| Strains | Habitat | Cultural characteristic in medium 79[(1)] | | | | NBP | Most similar sequences found in the Genbank | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GT | pH | Colony color | Ø[(2)] (mm) | | Species | SI (%) | Number of accession |
| *Mimosa tenuiflora*[(3)] | | | | | | | | | |
| UFLA 01-726 | Cerrado | FT | alcaline | white | 2 | 519 | *Burkholderia* sp. | 99 | FN543721 |
| UFLA 01-756 | Cerrado | FT | acid / alkaline | white | < 1 | 901 | *B. nodosa* | 99 | AY773192 |
| UFLA 01-733 | Cerrado | FT | alkaline | white | 1 | 678 | *Burkholderia* sp. | 99 | FN543723 |
| UFLA 01-753 | Cerrado | FT | alkaline | colorless | 1 | 1246 | *B. nodosa* | 99 | AY773192 |
| UFLA 01-748 | Cerrado | FT | alkaline | white | 1 | 725 | *Burkholderia* sp. | 100 | FN543681 |
| UFLA 01-731 | Peat bogs | FT | alkaline | white | 2 | 1272 | *Burkholderia nodosa* | 99 | AY773192 |
| UFLA 01-744 | Peat bogs | FT | Acid | colorless | 1 | 866 | *Burkholderia* sp. | 100 | FN543748 |
| UFLA 01-739 | Rocky outcrops | FT | Acid | yellow | 1 | 883 | *Burkholderia* sp. | 99 | FN543691 |
| UFLA 01-751 | Rocky outcrops | FT | Acid | colorless | 1 | 879 | *Burkholderia* sp. | 99 | FN543677 |
| UFLA 01-732 | Peat bogs | IT | acid / alkaline | white | 1 | 619 | *Burkholderia* sp. | 99 | FN543777 |
| *Macroptilium atropurpureum*[(3)] | | | | | | | | | |
| UFLA 04-248 | Cerrado | FT | alkaline | white | 1 | 710 | *Burkholderia* sp. | 99 | FN54379 |
| UFLA 04-269 | Cerrado | FT | alkaline | white | 1 | 839 | *Burkholderia* sp. | 99 | EU219864 |
| UFLA 04-405 | Rocky outcrops | FT | alkaline | white | 2 | 778 | *Burkholderia* sp. | 97 | AB366316 |
| UFLA 04-260 | Sandy bogs | FT | alkaline | colorless | 1 | 800 | *Burkholderia* sp. | 99 | FN543722 |

[(1)]GT - Growth time; FT - fast; IT - Intermediate. [(2)]Colony diameter (mm). NBP - Number of Base Pairs; SI (%) - Percentage of Similatiry in the GeneBank; [(3)]Host Plant

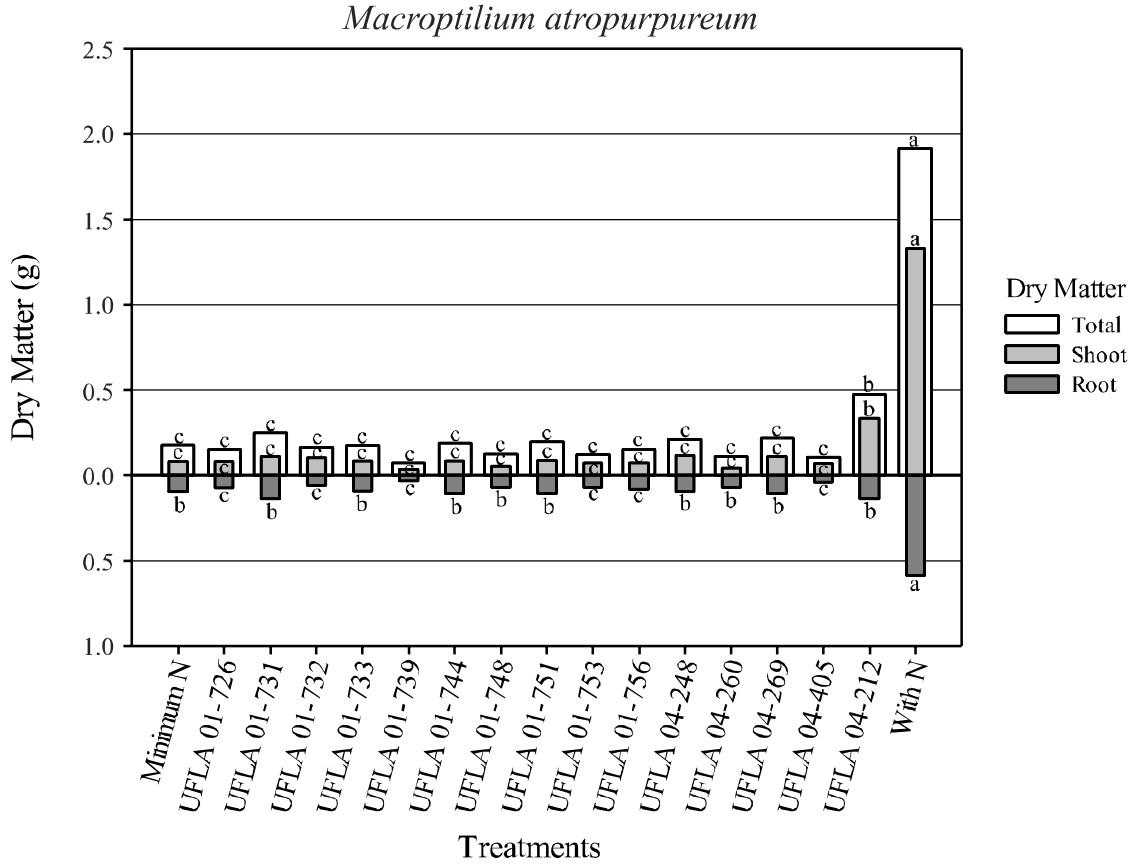
by using the statistical analysis program SISVAR version 5.3 (FERREIRA, 2011). The effects of the treatments were compared by the Scott-Knott test at 5% probability. NN data and NDM were transformed into square root of Y + 0.5. Graphs were obtained by the R software.

## RESULTS AND DISCUSSION

UFLA 04-212 (*Bradyrhizobium* sp.) was able to nodulate and promote growth of *M. atropurpureum* (Figura 1), which indicates that the experimental conditions were suitable for nodulation. However, of the 14 *Burkholderia* strains (Table 1), only UFLA 01-726 and UFLA 04-248 (14%) nodulated *M. atropurpureum*, but inefficiently, i.e., growth was similar to that of the control without inoculation. The species *M. atropurpureum* has been used as trap plant in studies on diversity since they capture bacteria of the alphaproteobacteria and betaproteobacteria classes (LIMA *et al.*, 2009). Although the interaction between *Burkholderia* and *M. atropurpureum* enable the formation of nodules (ANGUS *et al.*, 2013; ELLIOT *et al.*, 2007; LIMA *et al.*, 2009; MISHRA *et al.*, 2012), this interaction is not always considered effective (ANGUS *et al.*, 2013; BARRET; PARKER, 2005; MOULIN *et al.*, 2001). Angus *et al.* (2013) reported that *B. tuberum* (STM 678$^T$) favored the growth of *M. atropurpureum*. However, this effect was attributed to other processes that promote plant growth, since nodules were ineffective. Furthermore, there was no comparison with a positive control for nodulation and efficiency, but with an uninoculated control and with CIAT 899 (*Rhizobium tropici*) strain, which was inefficient.

Of the four strains isolated from *M. atropurpureum* nodules (Table 1), only one (UFLA 04-248) nodulated while re-inoculated in this legume. This behavior was

**Figure 1 -** Shoot dry matter, root dry matter and total dry matter (g), with coefficient of variation (CV) of 45.3, 23.3 and 31.4%, respectively, of *Macroptilium atropurpureum*, with the different treatments: treatments with individual inoculation with strains (Table 1 indicates the identification of strains); and two treatments without inoculation: one containing high mineral N concentration - 52.5 mg $L^{-1}$ (With N), and another containing low mineral N concentration- 5.25 mg $L^{-1}$ (Minimum N) concentration. All inoculated treatments received low mineral N concentration- 5.25 mg $L^{-1}$. Columns followed by the same letter do not statistically differ by the Scott-Knott test at 5% probability



also observed by other authors, when *Burkholderia fungorum* strains isolated from *M. atropurpureum* did not nodulate after re-inoculation in the same species (SILVA *et al*., 2012). However, *Burkholderia* was able to nodulate common bean (FERREIRA *et al*., 2012).

*Burkholderia* strains presented higher capacity of establishing symbiosis with the plants of the genus *Mimosa,* since 100% of the tested strains nodulated *M. bimucronata* species, and 86% nodulated *M. foliolosa* species (Tables 2 and 3). The four strains isolated from *M. atropurpureum* nodulated *M. bimucronata,* and three nodulated *M. foliolosa* (Tables 2 and 3). The present study corroborates other researches, which show higher affinity of *Burkholderia* strains with *Mimosa* genus. This affinity was indicated by the high frequency of *Burkholderia* strains isolated from *Mimosa* spp. located in different ecosystems (BARRET; PARKER, 2005; BONTEMPS *et al*., 2010; CHEN *et al.*, 2003, 2005, 2006, 2007, 2008; MISHRA *et al.*, 2012; REIS JÚNIOR *et al*., 2010). However, Elliot *et al.* (2007) found that the strain *Burkholderia tuberum* STM678$^{T}$ isolated from nodules of *Aspalathus carnosa* (MOULIN *et al*., 2001) failed to nodulate three species of *Mimosa* (*Mimosa pigra*, *M. diplotricha* and *M. pudica*), probably for presenting nodulation gene sequences different from those of nodulating *Burkholderia* species described, and which establish symbiosis with *Mimosa* spp. (GYANESHWAR *et al*., 2011).

In the experiment carried out with the species *M. bimucronata*, there was significant effects of treatments for most variables NN, NDM, SDM, plant height, diameter, and DQI (Table 2). The strains UFLA 01-732, UFLA 01-733, UFLA 01-739, UFLA 01-748, UFLA 01- 751, UFLA 01-756, UFLA 04-248 and UFLA 04-405 presented higher number of nodules, and were grouped with the inoculant strain for this culture (BR 3460) (Table 2).

These strains, except for UFLA 01-756, also had higher nodules dry matter production. The strains UFLA 01-744, UFLA 01-753, UFLA 04-260, UFLA 04-269, despite not presenting number of nodules similar to that of the inoculant strain (BR 3460), presented equivalent nodules dry matter production (Table 2). In relation to shoot dry matter production, the strains UFLA 01-731, UFLA 01-739, UFLA 01-748, UFLA 04-260, UFLA 04-

**Table 2** - Number of nodules (NN), nodules dry matter (NDM), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM), plant height and diameter in *Mimosa bimucronata* and Dickson quality index (DQI) in different treatments

| Treatments | NN | NDM | SDM | RDM | TDM | Height cm | Diameter mm | DQI |
|---|---|---|---|---|---|---|---|---|
| | | ---------- mg plant$^{-1}$ ---------- | | | | | | |
| 5.25 mg L$^{-1}$ N Without inoculation | 0 b | 0 b | 135 b | 71.3 a | 207.5 a | 12.67 b | 1.14 b | 0.01404 b |
| UFLA 01-726 +5.25 mg L$^{-1}$ N | 50.75 b | 3.7 b | 140 b | 73.8 a | 213.8 a | 12.76 b | 1.33 b | 0.01939 b |
| UFLA 01-731 +5.25 mg L$^{-1}$ de N | 55.62 b | 6.2 b | 256 a | 50 a | 303.8 a | 18.52 a | 1.56 a | 0.01816 b |
| UFLA 01-732 +5.25 mg L$^{-1}$ de N | 134 a | 11.2 a | 202 b | 86.3 a | 288.8 a | 14.92 a | 1.44 a | 0.02152 b |
| UFLA 01-733 +5.25 mg L$^{-1}$ de N | 130.75 a | 16.2 a | 171 b | 76.3 a | 248.8 a | 11.31 b | 1.14 b | 0.02116 b |
| UFLA 01-739 +5.25 mg L$^{-1}$ de N | 138.62 a | 15.0 a | 224 a | 102.5 a | 323.8 a | 14.95 a | 1.64 a | 0.02960 a |
| UFLA 01-744 +5.25 mg L$^{-1}$ de N | 53.62 b | 10.0 a | 144 b | 85.0 a | 227.5 a | 11.5 b | 1.43 a | 0.02482 a |
| UFLA 01-748 +5.25 mg L$^{-1}$ de N | 156.12 a | 18.7 a | 285 a | 87.5 a | 375.0 a | 18.26 a | 1.57 a | 0.02500 a |
| UFLA 01-751 +5.25 mg L$^{-1}$ de N | 119.87 a | 10.0 a | 206 b | 83.8 a | 287.5 a | 13.86 b | 1.28 b | 0.02011 b |
| UFLA 01-753 +5.25 mg L$^{-1}$ de N | 66.37 b | 11.2 a | 199 b | 87.5 a | 283.8 a | 13.21 b | 1.51 a | 0.02647 a |
| UFLA 01-756 +5.25 mg L$^{-1}$ de N | 104.12 a | 6.2 b | 194 b | 82.5 a | 275.0 a | 14.7 a | 1.51 a | 0.02387 a |
| UFLA 04-248 +5.25 mg L$^{-1}$ de N | 144 a | 12.5 a | 161 b | 80.0 a | 240.0 a | 12.76 b | 1.62 a | 0.02618 a |
| UFLA 04-260 +5.25 mg L$^{-1}$ de N | 31.75 b | 11.2 a | 242 a | 97.5 a | 340.0 a | 15.11 a | 1.55 a | 0.02675 a |
| UFLA 04-269 +5.25 mg L$^{-1}$ de N | 35.12 b | 10.0 a | 186 b | 78.8 a | 265.0 a | 12.78 b | 1.34 b | 0.02467 a |
| UFLA 04-405 +5.25 mg L$^{-1}$ de N | 123.87 a | 21.2 a | 265 a | 118.8 a | 383.8 a | 16.48 a | 1.69 a | 0.03110 a |
| BR-3460 +5.25 mg L$^{-1}$ de N | 89.75 a | 13.7 a | 207 b | 100.0 a | 306.3 a | 17.07 a | 1.55 a | 0.02387 a |
| 52.5 mg L$^{-1}$ de N Without inoculation | 0 b | 0 b | 295 a | 117.5 a | 412.5 a | 13.17 b | 1.58 a | 0.02549 a |
| CV (%) | 46.41 | 67.50 | 43.16 | 48.84 | 43.26 | 26.52 | 16.64 | 15.95 |

Table 1 shows the identification of strains that compose treatments with inoculation, and BR 3460 is the positive control. The two treatments without inoculation: one containing high mineral N concentration (52.5 mg L$^{-1}$) and another containing low mineral N concentration (5.25 mg L$^{-1}$) are the negative controls. Columns followed by the same letter do not present statistical differences by the Scott-Knott test, at 5% probability

405 were superior to the inoculant strain, and equivalent to the control with high mineral N concentration (Table 2). There were no differences between treatments in relation to root dry matter and total dry matter (Table 2).

In relation to the height of *M. bimucronata*, the strains UFLA 01-731, UFLA 01-732, UFLA 01-739, UFLA 01-748, UFLA 01-756, UFLA 04-260, UFLA 04-40 were similar to the strain BR 3460, which were superior to the control with high nitrogen concentration (Table 2). All these strains plus UFLA 01-744, UFLA 01-753, and UFLA 04-248 also promoted higher diameter growth, and were equivalent to the control with high mineral N concentration.

For the Dickson quality index (DQI), the strains UFLA 01-739, UFLA 01-744, UFLA 01-748, UFLA 01-753, UFLA 01-756, UFLA 04-248, UFLA 04-260, UFLA 04-269 and UFLA 04-405 were equivalent to the inoculant strain (BR 3460) and to the control with high N concentration (Table 2). However, the strains UFLA 01-739, UFLA 01-748 and UFLA 04-405 should be highlighted. Of the 14 tested strains, in the experiment carried out with *M. foliolosa*, only two did not nodulate this species, UFLA 01-731 and UFLA 04-269, which were isolated from *Mimosa tenuiflora* and *Macroptilium atropurpureum*, respectively (Table 3). *M. folilosa* is an endemic species of rupestrian grassland of Serra do Cipo, MG, and this is the first study that evaluates the nodulation and efficiency of nitrogen-fixing strains in its development; therefore, no strains have been approved by MAPA as inoculant yet.

The strains that presented higher number of nodules production were UFLA 01-726, UFLA 01-733, UFLA 01-748 and UFLA 04-248. With the exception of UFLA 01-733, these strains also promoted greater nodules dry matter production (Table 3).

All parameters showed significant effects; however, no strain was able to surpass, nor was equivalent to the results obtained with the control with high nitrogen concentration for shoot dry matter, total dry matter, height and diameter (Table 3). Root dry matter production by the strains UFLA 01-731, UFLA 01-733, UFLA 01-751, UFLA 04-260 and UFLA 04-269 was equivalent to that of the control with high mineral N concentration.

The values obtained by the Dickson quality index

**Table 3 -** Number of nodules (NN), nodules dry matter (NDM), shoot dry matter (SDM), root dry matter (RDM), and total dry matter(TDM), height and diameter of *Mimosa foliolosa* and Dickson quality index (DQI), in different treatments

| Treatments | NN | NDM | SDM | RDM | TDM | Height cm | Diameter mm | DQI |
|---|---|---|---|---|---|---|---|---|
| | | ---------- mg plant$^{-1}$ ---------- | | | | | | |
| 5.25 mg L$^{-1}$ de N Without inoculation | 0 d | 0 c | 207.2 b | 37.70 b | 245.0 b | 10.15 b | 1.4317 b | 0.01952 b |
| UFLA 01-726 +5.25 mg L$^{-1}$ de N | 24.75 a | 26.7 a | 245.1 b | 48.70 b | 293.9 b | 10.99 b | 1.5462 b | 0.02593 a |
| UFLA 01-731 +5.25 mg L$^{-1}$ de N | 0 d | 0 c | 164.4 c | 31.37 a | 195.7 c | 10.14 b | 1.0762 c | 0.01260 b |
| UFLA 01-732 +5.25 mg L$^{-1}$ de N | 16.37 b | 10.2 c | 132.2 c | 30.50 b | 161.6 c | 7.74 c | 1.1987 c | 0.01586 b |
| UFLA 01-733 +5.25 mg L$^{-1}$ de N | 28.00 a | 14.7 b | 137.7 c | 29.62 a | 167.4 c | 7.20 c | 1.1925 c | 0.01500 b |
| UFLA 01-739 +5.25 mg L$^{-1}$ de N | 12.12 c | 19.2 b | 197.9 b | 45.50 b | 243.4 b | 9.43 b | 1.4637 b | 0.01723 a |
| UFLA 01-744 +5.25 mg L$^{-1}$ de N | 3.62 d | 4.4 c | 166.2 c | 30.37 b | 196.6 c | 9.25 b | 1.1825 c | 0.01330 b |
| UFLA 01-748 +5.25 mg L$^{-1}$ de N | 26.50 a | 30.4 a | 276.1 b | 55.62 b | 331.7 b | 11.65 b | 1.6037 b | 0.02823 a |
| UFLA 01-751 +5.25 mg L$^{-1}$ de N | 6.38 c | 7.7 c | 209.5 b | 40.50 a | 253.0 b | 10.00 b | 1.6012 b | 0.02249 a |
| UFLA 01-753 +5.25 mg L$^{-1}$ de N | 1.43 d | 5.2 c | 166.0 c | 27.75 b | 193.7 c | 8.77 c | 1.2857 c | 0.01561 b |
| UFLA 01-756 +5.25 mg L$^{-1}$ de N | 6.00 c | 5.1 c | 201.1 b | 35.75 b | 236.9 b | 9.69 b | 1.4986 b | 0.01723 b |
| UFLA 04-248 +5.25 mg L$^{-1}$ de N | 29.00 a | 28.0 a | 196.6 b | 46.25 b | 242.9 b | 9.73 b | 1.2342 c | 0.02164 a |
| UFLA 04-260 +5.25 mg L$^{-1}$ de N | 3.12 c | 9.1 c | 166.2 c | 36.87 a | 255.9 b | 8.51 c | 1.4662 b | 0.02365 a |
| UFLA 04-269 +5.25 mg L$^{-1}$ de N | 0 d | 0 c | 140.9 c | 32.25 a | 173.1 c | 9.00 c | 1.0400 c | 0.01195 b |
| UFLA 04-405 +5.25 mg L$^{-1}$ de N | 12.87 c | 11.1 c | 184.0 b | 38.12 b | 250.7 b | 9.46 b | 1.3450 c | 0.01913 a |
| UFLA 01-750 +5.25 mg L$^{-1}$ de N | 21.12 b | 21.9 b | 194.9 b | 52.50 b | 247.4 b | 9.24 b | 1.4937 b | 0.02668 a |
| 52.5 mg L$^{-1}$ de N Without inoculation | 0 d | 0 c | 429.1 a | 68.62 a | 497.7 a | 16.55 a | 2.0650 a | 0.03462 a |
| CV (%) | 62.36 | 71.64 | 35.03 | 46.57 | 33.97 | 21.27 | 27.44 | 23.25 |

Table 1 shows the identification of strains that compose the inoculation treatments, and UFLA 01-750 is the positive control. The two treatments without inoculation: one containing high mineral N concentration (52.5 mg L$^{-1}$), and another containing low mineral N concentration (5.25 mg L$^{-1}$) are the negative controls. Columns followed by the same letter do not present statistical differences by the Scott-Knott test, at 5% probability

by the strains UFLA 01-726, UFLA 01-739, UFLA 01-748, UFLA 01-750, UFLA 01-751, UFLA 04-248, UFLA 04-260, and UFLA 04-405 were equivalent to that of the control with high N concentration (Table 3).

The *Mimosa* species evaluated this study are promising to be used in the recovery of degraded areas. The use of native legumes species that establish symbosis with nitrogen-fixing bacteria is of great interest for degraded areas restoration, since they established themselves in the field more quickly, and promote greater biomass accumulation and contribute to the improvement of soil fertility, favoring other species that are not able to establish this type of symbiosis (CHAER *et al*., 2011).

This study is the first to evaluate the potential of bacteria strains from nodules of legume species cultivated in rupestrian grasslands soils. The soils of this region are oligotrophic and acid (CARVALHO *et al*., 2012; 2014; NEGREIROS; MORAIS; FERNANDES, 2008), and the selection of strains adapted to these conditions may be used to obtain seedlings for the recovery of areas which have soils with these characteristics.

Although *Mimosa foliolosa* is an endemic species of the same region where the *Burkholderia* strains were obtained, they were more efficient in *Mimosa bimucronata*.

It can be considered that the strains UFLA 01-739, 01-748, 01-751, 04-260, 04-405 were in general the most effective in promoting the growth of *Mimosa* species. UFLA 01-739, 01-748, and 04-405 were considered potential inculants for *M. bimucronata;* and UFLA 01-751 and UFLA 04-260 were considered potential inoculants for *M. foliolosa*.

## CONCLUSIONS

1. *Burkholderia* strains establish symbiosis with the species *Macroptilium atropurpureum*, *Mimosa bimucronata* and *Mimosa foliolosa*, and present greater specificity for species of the genus *Mimosa*;
2. *Macroptilium atropurpureum* nodulation by *Burkholderia* strains occurred only with two of the 14 strains, and was inefficient;
3. *Burkholderia* strains isolated from *Macroptilium atropurpureum* and *Mimosa tenuiflora* cultivated in rupestrian grasslands soils promote plant growth of the species *Mimosa bimucronata* and *Mimosa foliolosa*, with potential to be used as inoculants for these species.
4. *Burkholderia* strains promote *Mimosa* spp. growth, but not *Macroptilium atropurpureum*.

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