Morphogenetic and structural characteristics of *Urochloa* species under inoculation with plant-growth-promoting bacteria and nitrogen fertilisation

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Abstract. Ineffective management of pastures still constitutes the main problem in the Brazilian livestock industry, in which recovery of soil fertility is a determinant for soil restoration. In this context and in combination with the adoption of sustainable technologies, the use of plant-growth-promoting bacteria (PGPB) may represent an alternative to the use of chemical fertilisers that would reduce costs and environmental impacts. In this study, morphogenetic and structural characteristics were evaluated in three *Urochloa* (syn. *Brachiaria*) varieties. *Urochloa* is a genus that is present in the most degraded pastures in Brazil. The three varieties were inoculated with five PGPB (*Azospirillum brasilense* Ab-V5 and Ab-V6, *Pseudomonas fluorescens* CCTB 03 and ET76, and *Pantoea ananatis* AMG521) and treated with different doses of nitrogen (N) fertiliser (0, 50 and 100 kg N ha\(^{-1}\)) in pots filled with sandy soil under greenhouse conditions. In general, for *Marandu grass* *U. brizantha* cvv. BRS Paiaguás and Xaraés, the best performances for leaf and stem elongation rates and number of basal tillers were obtained with strains CCTB 03 and AMG521. For *U. ruziziensis*, the best performance in the duration and rate of renewal of leaves and leaf senescence was observed with strains AMG521, Ab-V5 and Ab-V6. This result indicated the specificity of the effects of bacterial strains on different genotypes of *Urochloa*. Differences in the interactions between PGPB and N fertilisation were also verified. In *U. brizantha*, interaction effects were additive, whereas in *U. ruziziensis*, they were competitive. The results indicate the feasibility of inoculation of *Urochloa* with elite strains of PGPB that positively affect the production of forage biomass and allow reductions in N fertiliser usage.

Additional keywords: Congo grass, inoculation in forage, N mineral, rhizobacteria, sustainability in pasture.

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Introduction

Ineffective management of pastures constitutes the major problem for the Brazilian livestock industry. Poorly managed pastures established on soils of low fertility result in low stocking rates, low levels of weight gain, low forage biomass, and high chances of degradation. The scenario is particularly concerning in degraded areas occupied by pastures, which constitute ~126 Mha in Brazil (Hungria *et al*. 2016). In this context, the adoption of environmentally sustainable strategies that maximise forage mass production is essential.

The composition of the soil and the nutrient supply to plants (especially nitrogen, N) are factors that determine the restructuring of the productivity of a pasture (Hungria *et al*. 2016). However, the supply of nutrients through mineral fertilisation has been a point of contention because it is associated with negative environmental impacts such as the eutrophication of waters (rivers, lakes, ground water) and the emission of greenhouse gases (GHGs) (Ormeño-Orrillo *et al*. 2013; Sá *et al*. 2017).

Awareness of the importance of adopting sustainable technologies is growing in agriculture, with emphasis on the use of plant-growth-promoting bacteria (PGPB). These bacteria promote the growth of plants through several mechanisms, including biological N\(_2\) fixation (BNF), production of phytohormones, and induction of tolerance to biotic and abiotic stresses; these mechanisms may even act in
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combination (Bashan and de-Bashan 2010; Hungria et al. 2010; Bashan et al. 2014). The most extensively studied PGPB have been rhizobia in the case of BNF with legumes, and bacteria of the genus Azospirillum in the case of grass. However, in Brazil, there have been few studies on PGPB in forage grasses.

Leite et al. (2018) evaluated the increase in productivity, reduction in the N fertiliser used, and mitigation of water stress by inoculation of Marandu grass (Urochloa brizantha (Hoehst. ex A.Rich.) R.D.Webster; syn. Brachiaria brizantha (A.Rich.) Staff) with Azospirillum brasilense; they found that inoculation with this bacterium guaranteed greater plant height, and tiller and root mass, especially in dry conditions. In relation to the production of forage mass, the authors observed that inoculation with A. brasilense increased annual yield by 14%. Aguirre et al. (2018) concluded that the use of A. brasilense promoted a reduction in N-fertiliser use to ~20% in Cynodon dactylon (L.) Pers. cv. Coastcross-1 pasture without compromising forage mass production.

Another determinant in the success of pastures is the understanding of morpho-physiological mechanisms and their interactions with the environment (Chapman and Lemaire 1993). With this information, strategies can be adopted that alter the dynamics of biomass accumulation, because changes in morphogenesis cause changes in the structure of the plant canopy, such as the size of the leaves, stems and tillers (Martucello et al. 2011).

The objective this study was to evaluate the morphogenetic and structural characteristics of three Urochloa varieties inoculated with different PGPB and receiving different doses of N fertiliser.

Materials and methods

Experimental details

The experiment was performed in protected environments in greenhouses from October 2015 to October 2016 at the Technical Centre of Irrigation, State University of Maringá, Maringá, PR, Brazil (23°25’S, 51°57’W; average elevation 542 m a.m.s.l.). According to Köppen, the climate type is Cfa mesothermal moist, with abundant rains in the summer and dry winters.

The treatments consisted of a non-inoculated control and five PGPB: Azospirillum brasilense Ab-V5 (= CNPSo 2083) and Ab-V6 (= CNPSo 2084), Pseudomonas fluorescens CCTB 03 (CNPSo = 2719) and ET76 (= CNPSo 2799), and Pantoea ananatis AMG521 (= CNPSo 2798). Each inoculated treatment was studied at three doses of N fertiliser (0, 50 and 100 kg N ha⁻¹) as urea.

The strains were placed in the Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja (World Federation Culture Collection, WFC #1213; World Data Centre for Microorganisms, WDCM #1054). The bacteria were derived as follows: A. brasilense Ab-V5 and Ab-V6, selected in Brazil, initially for maize (Zea mays L.) and wheat (Triticum aestivum L.), from PGPB selection programs of Embrapa Soja (Hungria et al. 2010); P. fluorescens CCTB 03 from the company Total Biotecnologia (Curitiba, PR, Brazil); and P. fluorescens ET76 and P. ananatis AMG521 from the University of Seville (the former isolated in Morocco, Aarab et al. 2016; the latter isolated in Spain, Megías et al. 2016). For preparation of inoculant, the strains were grown in DYGS medium (Fukami et al. 2018), and the concentration was adjusted to 10⁸ cells mL⁻¹, which was obtained by correlation of the growth curves previously obtained by the Culture Collection for each strain and the corresponding optical density.

The treatments were evaluated in three forage varieties of Urochloa: Xaraés grass (U. brizantha cv. Xaraés); Paiaguás grass (U. brizantha cv. BRS Paiaguás); and Congo grass (Urochloa ruziensis (Germ. & C.M.Evrard) Crins, syn. Brachiaria ruziensis Germ. & C.M.Evrard). The test was completely randomised in a 3 × 6 factorial design with four replications totalling 72 experimental plots for each of the three forages (i.e. n = 72 per forage).

The assays were carried out in 15-kg pots filled with 15 dm³ of Ferralsol soil with a sandy texture and the following chemical characteristics: pH 4.7, calcium 1.0 cmol dm⁻³, magnesium 0.5 cmolc dm⁻³, aluminium 0.1 cmolc dm⁻³, Mehlich phosphorus (P) 5.0 mg dm⁻³, potassium (K) 0.16 cmolc dm⁻³, base saturation 32%, and organic matter 2%. All pots received the equivalent of 20 kg N ha⁻¹ (urea 45% N), 84 kg K₂O ha⁻¹ (potassium chloride, 48% K₂O) and 42.5 kg P₂O₅ ha⁻¹ (simple superphosphate, 18% P₂O₅) incorporated into the soil at sowing (Martha Junior et al. 2004). In order to simulate real conditions in the areas of pastures, the pH of the soil was not corrected.

Bacterial inocula were prepared in liquid medium specific for each species and, at the time of sowing, adjusted to the final concentration of 10⁸ cells mL⁻¹. Then, each inoculum was mixed with soil (15 mL kg⁻¹) and left to dry in the shade for 30 min; 10 g was sown per pot of each forage species. One week after emergence of seedlings, they were thinned, leaving five plants per pot.

Immediately after thinning, N fertiliser was applied, with treatments corresponding to 100 and 50 kg N ha⁻¹. For 100 kg N ha⁻¹, 0.75 g N was added per pot (1.67 g urea pot⁻¹) and for 50 kg N ha⁻¹, 0.375 g N was added per pot (0.83 g urea pot⁻¹), split into two doses and incorporated into the soil contained in the pot. Half the dose was applied soon after the thinning, and the other half was applied 28 days later. The control received no N fertiliser after thinning.

Plants were irrigated three times daily on mild days and four times daily on high-temperature days by automated irrigation with sterile distilled water for 2 min to achieve field capacity. Maximum and minimum temperature data were recorded in the greenhouse during the study period (Fig. 1).

Evaluations

When the plants reached, on average, 35 cm in height, shoots were cut to 15 cm. Height measurements were performed three times each week by using a ruler with 1-mm increments. Morphogenic and structural characterisation were evaluated weekly on two tillers in each pot, marked by coloured wire, totalling 53 evaluations throughout the experimental period. The lengths of the green leaves and pseudostem were measured by means of a ruler with 1-mm increments. For expanding leaves, the measurement was from the ligule of the last expanded leaf as a reference. Expanded leaves were measured from the ligule to the tip of the green leaf. The length of the pseudostem was obtained as the distance from
the ground to the ligule of the youngest completely expanded leaf. Lengths of expanded leaves, cut leaves and dead leaves were also recorded. These measures were used to determine the following rates according to Sbrissia and da Silva (2008):

- **Leaf appearance rate (LAR):** ratio of number of leaves per tiller appearing in the evaluated period to number of days in the period.
- **Phyllochron (Phyllo):** number of days in which two leaves grow on the same tiller.
- **Leaf elongation rate (LER):** ratio of total elongation of all leaf blades (cm) to number of days in the evaluation period (i.e. \((\text{final length} - \text{initial length})/\text{no. of days counted}\)).
- **Leaf senescence rate (LSR):** mean variation in length of the senescent portion of the leaf, which was obtained as the product of length of the senescent leaf blade and proportion of corresponding senescent tissue observed throughout the evaluation period.
- **Number of live (non-senescent) leaves (NLL).**
- **Duration of life of leaves (DLL):** number of live leaves \(\times\) Phyllo.
- **Stem elongation rate (SER),** difference in the length of the pseudostem between the end and the beginning of the experimental period divided by the number of days (i.e. (final length – initial length)/no. of days counted).

Tiller population density was obtained by counting the tillers in each pot. This was done every 28 days, and the tillers were classified as basal tillers or aerial tillers, in accordance with the node that gave rise to it.

### Statistical analyses

Data for each forage were analysed separately for each of the six inoculation treatments \(\times\) three levels of N fertiliser. The means of the control group (without bacteria or without fertilisation) and the treated groups were compared by using Dunnett’s test, and among treatments, means were compared by Tukey’s test. When an effect of the interaction among factors was identified, the responses to each type of bacteria were compared within each level of N fertilisation. In all statistical analyses, PROC GLM of the statistical package SAS version 9.2 (SAS Institute, Cary, NC, USA) was used with a significance level of \(P = 0.05\).

### Results

Inoculation with PGPB and doses of N fertiliser had an effect on LER and number of basal tillers in Paiaguás grass
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(Table 1). Plants inoculated with the bacteria *P. fluorescens* CCTB 03 and *P. ananatis* AMG521 (at nil N) showed higher LER, leading to increases of >100% in the elongation of leaves in relation to those in the non-inoculated treatment. No differences were observed for the LER parameter at the dose 50 kg N ha\(^{-1}\). At the dose 100 kg N ha\(^{-1}\), inoculation with *P. ananatis* AMG521 provided an increase in LER of 62% compared with the N treatment alone. At this dose, inoculation with *A. brasilense* Ab-V6, *P. fluorescens* CCTB 03 and *P. fluorescens* ET76 increased LER by 36%, 54% and 51%, respectively, compared with that in the N treatment alone (Table 1).

As seen with LER, inoculation with *P. fluorescens* CCTB 03 and *P. ananatis* AMG521 without N promoted an increase of >100% in the number of basal tillers compared with the other treatments, except for *P. fluorescens* ET76, which resulted in a 42% increase over the non-inoculated control (Table 1). At the dose 50 kg N ha\(^{-1}\), no significant increases due to inoculation were observed for the number of basal tillers; however, the treatments inoculated with *A. brasilense* Ab-V5 and Ab-V6 had lower numbers of basal tillers than the non-inoculated treatment (Table 1). At the dose 100 kg N ha\(^{-1}\), inoculation with *P. ananatis* AMG521 resulted in a greater number of basal tillers in plants than the non-inoculated treatment; inoculation with the two strains of *P. fluorescens* produced results similar to that found with *P. ananatis* AMG521. The treatment with *P. ananatis* AMG521 and 100 kg N ha\(^{-1}\) increased the number of basal tillers per pot by 22% compared with the N treatment alone.

There were N-fertiliser effects on LAR, Phyllo and DLL in Paiaguás grass. The dose 100 kg N ha\(^{-1}\) provided higher LAR and lower Phyllo and DLL than those in other treatments (Table 2).

Among PGPB, *P. fluorescens* CCTB 03 provided the highest SER in Paiaguás grass (Table 3), with an increase of 62% compared with the non-inoculated treatment. However, for other characteristics, no differences were found among the treatments.

For Xaraés grass, an N fertilisation × PGPB interaction was identified for LER, LSR and number of basal tillers (Table 4). In the absence of N fertilisation, *P. fluorescens* ET76 and *P. ananatis* AMG521 promoted increases of 51% and 52%, respectively, in LER compared with the non-inoculated treatment. The other strains, *P. fluorescens* CCTB 03 and *A. brasilense* Ab-V5 and Ab-V6, also resulted in longer upper leaves than those in the non-inoculated treatment, by 48%, 43% and 34%, respectively. When associated with the dose 50 kg ha\(^{-1}\) N, no strain promoted an increase in LER in relation to the non-inoculated treatment, and there was a decrease in LER for *A. brasilense* Ab-V6 compared with the non-inoculated treatment. Finally, at 100 kg N ha\(^{-1}\), the LER in the treatment with *P. fluorescens* CCTB 03 was significantly higher than that of the non-inoculated control (16%) and the *A. brasilense* Ab-V5 treatment (18%) (Table 4).

In the absence of N fertiliser, inoculation with *P. fluorescens* CCTB 03 increased the renewal of tissues (LSR) by >100% compared with the non-inoculated control; this effect was not observed under inoculation with the other bacteria (Table 4). When associated with application of 50 kg N ha\(^{-1}\), no treatment differed from the non-inoculated control. Finally, at the highest dose of 100 kg N ha\(^{-1}\), only *P. fluorescens* ET76 deceased LSR; there was no difference between the remaining strains and the non-inoculated treatment (Table 4).

In the absence of N fertiliser, the number of basal tillers of Xaraés grass increased by 54% under inoculation with *P. ananatis* AMG521 compared with the non-inoculated control (Table 4). Significant increments were also observed

### Table 2. Stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (LLN) and leaf senescence rate (LSR) of *Urochloa brizantha* cv. BRS Paiaguás fertilised with different doses of nitrogen (N) fertiliser

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment (kg N ha(^{-1}))</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SER (cm day(^{-1}))</td>
<td>0.73a</td>
<td>0.72a</td>
</tr>
<tr>
<td>LAR (no. of leaves day(^{-1}))</td>
<td>0.11b</td>
<td>0.10b</td>
</tr>
<tr>
<td>Phyllo (no. of days leaf(^{-1}))</td>
<td>9.98a</td>
<td>10.34a</td>
</tr>
<tr>
<td>DLL (days)</td>
<td>47.3a</td>
<td>46.6a</td>
</tr>
<tr>
<td>LLN (no. of live leaves tiller(^{-1}))</td>
<td>4.87a</td>
<td>4.70a</td>
</tr>
<tr>
<td>LSR (cm day(^{-1}))</td>
<td>0.82a</td>
<td>0.71a</td>
</tr>
</tbody>
</table>

### Table 3. Stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (LLN) and leaf senescence rate (LSR) of *Urochloa brizantha* cv. BRS Paiaguás inoculated with different plant-growth-promoting bacteria

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Not inoculated</th>
<th><em>Azospirillum brasilense</em></th>
<th><em>Pseudomonas fluorescens</em></th>
<th><em>Pantoea ananatis</em> AMG521</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab-V5</td>
<td>Ab-V6</td>
<td>CCTB 03</td>
<td>ET76</td>
</tr>
<tr>
<td>SER (cm day(^{-1}))</td>
<td>0.62b</td>
<td>0.81b</td>
<td>0.66b</td>
<td>0.94a*</td>
</tr>
<tr>
<td>LAR (no. of leaves day(^{-1}))</td>
<td>0.11a</td>
<td>0.12a</td>
<td>0.11a</td>
<td>0.12a</td>
</tr>
<tr>
<td>Phyllo (no. of days leaf(^{-1}))</td>
<td>9.55a</td>
<td>8.83a</td>
<td>10.00a</td>
<td>8.88a</td>
</tr>
<tr>
<td>DLL (days)</td>
<td>45.26a</td>
<td>39.62a</td>
<td>43.04a</td>
<td>42.32a</td>
</tr>
<tr>
<td>LLN (no. of live leaves tiller(^{-1}))</td>
<td>4.79a</td>
<td>4.78a</td>
<td>4.46a</td>
<td>4.98a</td>
</tr>
<tr>
<td>LSR (cm day(^{-1}))</td>
<td>0.79a</td>
<td>0.95a</td>
<td>1.18a</td>
<td>0.92a</td>
</tr>
</tbody>
</table>
Table 4. Leaf elongation rate (LER), leaf senescence rate (LSR) and number of basal tillers of *Urochloa brizantha* cv. Xaraés inoculated with different plant-growth-promoting bacteria under different doses of nitrogen (N) fertiliser

| N rate (kg N ha⁻¹) | Not inoculated | Azospirillum brasilense | Pseudomonas fluorescens | Pantoea ananatis | CV (%)
<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ab-V5</td>
<td>Ab-V6</td>
<td>CCTB 03</td>
<td>ET76</td>
</tr>
<tr>
<td>0</td>
<td>1.60c</td>
<td>2.37ab</td>
<td>2.14b</td>
<td>2.29ab</td>
<td>2.41a</td>
</tr>
<tr>
<td>50</td>
<td>2.21ab</td>
<td>1.93bc</td>
<td>1.83c</td>
<td>2.25ab</td>
<td>2.43a</td>
</tr>
<tr>
<td>100</td>
<td>3.11b</td>
<td>3.05b</td>
<td>3.37ab</td>
<td>3.61a</td>
<td>3.48ab</td>
</tr>
</tbody>
</table>

Values are means of four repetitions. Within rows, means followed by the same letter are not significantly different according to *t*-tests at *P* = 0.05. CV, Coefficient of variation.

Table 5. Stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (LLN) and leaf senescence rate (LSR) in *Urochloa brizantha* cv. Xaraés receiving different doses of nitrogen (N) fertiliser

<table>
<thead>
<tr>
<th>Parameter (cm day⁻¹)</th>
<th>Treatment (kg N ha⁻¹)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SER</td>
<td>0.36a</td>
<td>0.37a</td>
</tr>
<tr>
<td>LAR (no. of leaves day⁻¹)</td>
<td>0.07b</td>
<td>0.06c</td>
</tr>
<tr>
<td>Phyllo (no. of days leaf⁻¹)</td>
<td>14.90b</td>
<td>15.50a</td>
</tr>
<tr>
<td>DLL (days)</td>
<td>58.90b</td>
<td>66.70a*</td>
</tr>
<tr>
<td>LLN (no. of live leaves tiller⁻¹)</td>
<td>4.05b</td>
<td>4.27a*</td>
</tr>
<tr>
<td>LSR (cm day⁻¹)</td>
<td>1.20a</td>
<td>1.27a</td>
</tr>
</tbody>
</table>

Table 6. Leaf elongation rate (LER), stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (LLN), leaf senescence rate (LSR) and number of basal tillers of *Urochloa ruziensis* (Congo grass) receiving different doses of nitrogen (N) fertiliser

<table>
<thead>
<tr>
<th>Parameter (cm day⁻¹)</th>
<th>Treatment (kg N ha⁻¹)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LER</td>
<td>1.71b</td>
<td>1.60b</td>
</tr>
<tr>
<td>SER (cm day⁻¹)</td>
<td>0.87b</td>
<td>0.84b</td>
</tr>
<tr>
<td>LAR (no. of leaves day⁻¹)</td>
<td>0.12b</td>
<td>0.12b</td>
</tr>
<tr>
<td>Phyllo (no. of days leaf⁻¹)</td>
<td>8.94b</td>
<td>8.36ab</td>
</tr>
<tr>
<td>DLL (days)</td>
<td>48.02b</td>
<td>50.42b</td>
</tr>
<tr>
<td>LLN (no. of live leaves tiller⁻¹)</td>
<td>5.67a</td>
<td>6.11a</td>
</tr>
<tr>
<td>LSR (cm day⁻¹)</td>
<td>0.64c</td>
<td>1.36a*</td>
</tr>
<tr>
<td>Basal tillers (no. plant⁻¹)</td>
<td>23.50a</td>
<td>24.50c</td>
</tr>
</tbody>
</table>

for inoculation with strains *P. fluorescens* CCTB 03 (42%) and ET76 (24%). At the dose 50 kg N ha⁻¹, treatments inoculated with *A. brasilense* Ab-V5 and Ab-V6 had more tillers than the non-inoculated control. At the dose 100 kg N ha⁻¹, there was a reduction in basal tillers by inoculation with *A. brasilense* Ab-V6 compared with the non-inoculated control; other inoculated treatments were statistically similar to the control (Table 4).

There were differences among N-fertiliser doses with respect to LAR, Phyllo, DLL and LLN in Xaraés grass (Table 5). The dose 50 kg N ha⁻¹ increased Phyllo, LLs and LLN by 4%, 13% and 6%, respectively, over the nil-N rate. The highest LAR occurred at 100 kg N ha⁻¹. No differences were observed among the inoculated treatments and the non-inoculated control for SER, LAR, Phyllo, LLS, LLN or LSR in Xaraés grass.

Differences were observed among N-fertiliser doses for LER, SER, LAR, Phyllo, DLL, LLN and LSR in Congo grass (Table 6). The highest rate (100 kg N ha⁻¹) differed significantly from 0 kg N ha⁻¹ in all parameters assessed, except for number of basal tillers. The 100 kg N ha⁻¹ treatment was superior in all parameters except LLN. At the dose 50 kg N ha⁻¹, only LSR was higher than in the nil-N treatment (Table 6).

An effect of inoculation with the PGPB was verified for DLL and LSR in Congo grass (Table 7). Inoculation with *A. brasilense* Ab-V5 and *P. ananatis* AMG521 resulted in higher LSR than in the non-inoculated treatment, possibly due to the rapid development of tillers (Table 7).

Discussion

The benefits observed in LER, SER, LSR, DLL and number of basal tillers with the use of PGPB could result from increased production of growth hormones such as auxins, cytokinins and...
gibberellins that are synthesised by several of these bacteria (Pereira et al. 2015). Fukami et al. (2017) found that the metabolites produced by certain types of bacteria, such as auxins, gibberellins and their precursors, affected plant growth because these substances are responsible for several physiological events that result in rapid growth. According to Taiz and Zeiger (2013), the activity of auxin and gibberellin can modify cellular expansion, change the way the cell wall is expanded, modify stem and leaf elongation, and extend the duration of the life of these organs.

Fukami et al. (2017) described the synthesis of indole-3-acetic acid (IAA), indole-3-ethanol and lactic acid-3-indole by strains of *Azospirillum brasilense* Ab-V5 and Ab-V6. There are also reports of synthesis of IAA by *Pantoea ananatis* AMG521 (Megías et al. 2016). According to Pan et al. (1999), high production of plant hormones, particularly IAA, results in an increase in leaf elongation after promoting the growth and development of the plant organs. Moreover, IAA has the ability to change photosynthesis and the biosynthesis of some metabolites and other phytohormones, such as cytokinins and gibberellins (Ilyas and Bano 2010).

Figueiredo et al. (2010), evaluating IAA production by bacteria associated with *Urochloa* spp., reported that of 80 studied bacteria, 91% produced IAA, emphasising the impact that PGPB may have on the recovery of pasture. In corn, Zucarelli et al. (2011) observed greater growth of plants inoculated with *P. fluorescens* and attributed this effect to the large inflow of cytokinin to plants. According to Queiróz et al. (2006), *P. fluorescens* promotes plant growth through the increased production and concentration of plant hormones that influence plant physiological processes. Zucarelli et al. (2011), when assessing the effect of *P. fluorescens* on the growth rate of corn, concluded that the bacterium promoted increases in stem elongation rates and number of leaves. In the case of the ET76 strain of *P. fluorescens*, it is noteworthy that it was isolated from the rhizosphere of rice and presents other growth-promoting properties such as the ability to solubilise phosphate, in addition to having great potential as a biocontrol agent for disease prevention (Aarab et al. 2016).

The increased resistance to stress factors provided by the use of bacteria may have prolonged the duration of the life of plant leaves and resulted in a greater number of basal tillers, ensuring the persistence and survival of the grass for a longer period. In this sense, Cohen et al. (2015) found that strains of PGPB can secrete abscisic acid (ABA), which is involved in plant defence mechanisms against environmental stresses (Bauer et al. 2013). Cohen et al. (2009) inoculated maize plants with *A. lipoflavus* and found high levels of ABA, which increased the plant’s tolerance of drought. According to those authors, this tolerance to environmental stress provided by *Azospirillum* can be related to ABA and to prolines and polyamines. Polyamines (cadaverine, spermine and spermidine) are organic polymers that are associated with root growth and the suppression of plant stresses (Gupta et al. 2013). When inoculating seedlings of rice with *A. brasilense*, Cassán et al. (2009) found that the production of cadaverine increased root growth and reduced osmotic stress. In maize inoculated with *A. brasilense*, Rodriguez-Salazar et al. (2009) observed greater resistance to drought and, consequently, increased biomass production in the inoculated plants. Regarding the *A. brasilense* strains Ab-V5 and Ab-V6 used in this study, an increase in the expression of genes related to stress tolerance in maize was also observed under inoculation with these strains or their metabolites via seed or foliar application (Pereira et al. 2015; Fukami et al. 2017; Leite et al. 2018).

Another mechanism of action of PGPB, which possibly provided improved morphogenetic and structural responses in the forage plants evaluated, was BNF. Bulo and Döbereiner (1975) were pioneers in noting that some bacteria, identified as *Azospirillum*, present in the rhizosphere of forage grasses were able to fix N2, opening up new perspectives on the use of forages in the tropics fertilised with smaller quantities of N. More recently, inoculation with strains Ab-V5 and Ab-V6 of *A. brasilense* benefited the production of forage mass of two species of *Urochloa*, with increases of 5.4–22.1% (Hungria et al. 2016). These results may be related to the mode of action of the genus *Azospirillum*, both by BNF and by the production of phytohormones, which in this case change the morphology of the roots and allow greater exploitation of the soil and better absorption of nutrients (Iniguez et al. 2004).

Among the factors that influence the morphogenetic and structural characteristics of forage grasses, N provides the
greatest number of modifications. In this sense, Ferro et al. (2015) described that changes in intercepted light, water level and N fertilisation influenced by LAR, SER, DLL and LSR. Nitrogen, when available to plants, increases the development of new tissues, explaining the increments observed in the N-fertiliser treatments. Martinsello et al. (2011) observed that, under favourable conditions, N is quickly used by the plant, increasing the elongation and appearance of new leaves.

In general, it was evident that inoculation with bacteria associated only with the lowest level of N fertilisation (initial application of 20 kg N ha\(^{-1}\) at sowing, no N fertiliser treatment after thinning) promoted leaf and stem elongation, foliar appearance, leaf life and tillering. This would result in a reduction in the recommended dose of N fertiliser for plant formation and maintenance without compromising the forage mass production. Likewise, Aguirre et al. (2018), evaluating the inoculation of \textit{A. brasilense} strains on pasture of the genus \textit{Cynodon}, observed through a regression analysis that the use of inoculants allowed an estimated reduction of 20% in N fertiliser.

**Conclusion**

Inoculation with PGPB represents a viable and sustainable alternative for the growth and development of \textit{Urochloa}, because it led to growth promotion in three cultivars. Among the PGPB strains used, \textit{P. ananatis} AMG521 was effective for both \textit{Urochloa} species, \textit{P. fluorescens} CCTB 03 was effective for \textit{U. brizantha}, and \textit{A. brasilense} Ab-V5 and Ab-V6 were effective for \textit{U. ruziizensis}. There was also a difference in the interaction effects between PGPB and N fertilisation. In \textit{U. brizantha}, the effects were additive, whereas in \textit{U. ruziizensis}, they were competitive.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Author contributions**


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