



## Different methods of inoculation of nitrogen-fixing bacteria (*Azospirillum*) specific of grasses in sorghum

### Diferentes métodos de inoculação de bactérias fixadoras de nitrogênio (*Azospirillum*) específicas de gramíneas em sorgo

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#### Resumo

O sorgo é uma das culturas de grande relevância na produção de grãos na região do cerrado. Tem como principal uso na alimentação animal e é considerada uma ótima alternativa para a alimentação humana na forma de grãos. Uma alternativa promissora é o uso de bactérias fixadoras de nitrogênio (N) na cultura do sorgo. A inoculação com bactérias fixadoras de N, como *Azospirillum*, é uma alternativa para reduzir custos ao substituir o alto custo de produção devido à fertilização com nitrogênio. O objetivo deste trabalho foi avaliar diferentes métodos de inoculação de bactérias fixadoras de nitrogênio (*Azospirillum*) em cultura de sorgo. O experimento foi realizado na cidade de Goianésia, Goiás, Brasil. O delineamento experimental foi em blocos casualizados com cinco tratamentos, utilizando 4 repetições por tratamento, dispostos em diferentes métodos de inoculação de *Azospirillum* brasileiro em sorgo: inoculação testemunha, tratamento de sementes, aplicação em sulco, aplicação em cobertura após semeadura e aplicação 10 dias após a

emergência. Para determinar o crescimento e desenvolvimento, foram feitas as seguintes avaliações: espessura do caule, comprimento da raiz, volume da raiz, número de nós, massa fresca da parte aérea e massa seca da parte aérea. A inoculação das sementes apresentou superioridade em relação aos demais tratamentos quanto ao comprimento da raiz, número de nós, massa verde e massa seca. A inoculação com *Azospirillum* promoveu efeitos positivos na espessura da parte aérea e comprimento da raiz, obtendo quantidades significativas e inoculação das sementes com os melhores resultados.

#### Abstract

Sorghum is one of the crops of great relevance in the production of grains in the cerrado region. Its main use for animal feeding and is considered a great alternative for human food in the form of grains. A promising alternative is the use of nitrogen-fixing bacteria (N) in the sorghum crop. Inoculation with N-fixing bacteria, such as *Azospirillum*, is an alternative to reduce costs by replacing the high cost of production due to nitrogen fertilization. The objective of this work was to evaluate different methods of inoculating nitrogen - fixing bacteria (*Azospirillum*) in sorghum culture. The experiment was carried out in the city of Goianésia, Goiás, Brazil. The experimental design was randomized blocks with five treatments, using 4 replicates per treatment, arranged in different methods of inoculation of *Azospirillum* brasileiro in sorghum: control inoculation, seed treatment, application in furrow, application in cover after sowing and application 10 days after emergence. To determine growth and development, the following evaluations were made: stem thickness, root length, root volume, number of nodes, fresh shoot mass and shoot dry mass. Seed inoculation presented superiority in relation to other treatments regarding root length, number of nodes, green mass and dry mass. The inoculation with *Azospirillum* promoted positive effects on shoot thickness and root length, obtaining significant amounts and seed inoculation with the best results..

## INTRODUCTION

Sorghum is one of the crops of great relevance in the production of grains in the Cerrado biome. Its main use for animal feeding and is considered a great alternative for human food in the form of grains (DA SILVA et. al., 2009; LEAL et. al, 2013).

Cultivated since the origin of agriculture, sorghum has increased its space in the world grain market due to its following characteristics: adaptability, versatility and rusticity (DOS SANTOS et. al., 2015). In Brazil, according to Mariano & Sousa (2007), the culture is widely used in animal feeding, but several published works indicate its feasibility and the physiological potential to be used in energy production.

According to CONAB (2018), the Brazilian production of sorghum for the 2017/18 harvest is estimated at 1786.6 thousand tons, and the state of Goiás is the representative of 779.3 thousand tons of this amount. The CONAB (2018), still states that the planted area is estimated 632.8 thousand hectares and a productivity of 2823 kg/ha.

Sorghum is suitable for ensiling due to its phenotypic characteristics that may facilitate planting, management, harvesting and storage (SANTOS et al., 2013). It can also be cited for its great nutritional value and soluble carbohydrate concentration, important for good lactic fermentation, and its high dry matter yield per unit area (NEUMANN et al., 2002). This culture is very demanding in the nutritional aspects, especially in relation to the nitrogen, that can limit the productivity of grains (CASTILHOS et al., 2005)

Nitrogen-fixing bacteria are organisms that have the ability to establish symbiotic relationships with plants, colonizing the interior of plant tissues without causing symptoms of plant diseases and fixing N<sub>2</sub> present in the atmosphere (MOREIRA et al., 2013). According to Baldani et al. (1997), those are capable of forming symbiotic associations with grasses are considered facultative endophytes (BALDANI et al.,

1999). According to Moreira (2013), the genus *Azospirillum* stands out among the most studied; and also complements that the diazotrophic endophytic microorganisms of the genus play an important role in the rehabilitation and sustainability of the ecosystems, since they incorporate the nitrogen through the biological fixation.

The diversity of bacteria is directly related to the associated plant species (BALDANI et al., 1999), the association with *Azospirillum* spp. in the roots and soil of other grasses, such as maize, which registered values higher than 10<sup>6</sup> g<sup>-1</sup> soil bacteria (VERMA et al, 2012; CARDOZO, 2017) e and in the rhizosphere of brachiaria species in Goiás and Minas Gerais (10<sup>3</sup> a 10<sup>7</sup> bacteria g<sup>-1</sup> ) (REIS JR et al., 2000), besides rice, sorghum, forage grasses (FIGUEREIDO et al., 2008) and superficially disinfested roots (MOREIRA et. al., 2013).

Inoculation with nitrogen-fixing bacteria, such as *Azospirillum*, is an alternative to reduce costs by replacing the high cost of production due to nitrogen fertilization, favoring directly the production of phytohormones, in particular auxins, biological nitrogen fixation and solubilization of phosphates (CORREIA FILHO et al., 2018).

The objective of this work was to evaluate different methods of inoculation of nitrogen - fixing bacteria (*Azospirillum*) in sorghum culture.

## MATERIALS AND METHODS

The experiment was carried out at the experimental campus of the Evangelical Faculty of Goianésia, situated at 15°19 '22.32' 'S and 49° 08' 19.85" W in the city of Goianésia, Goiás, Brazil. According to Koppen (1931), as seasonal tropical (Aw), being characterized by two well defined seasons (dry and rainy), as well as the occurrence of drought periods during the rainy season.

The experimental design was randomized blocks with five treatments, using 4 replicates per treatment, arranged in different inoculation methods of *Azospirillum brasilense* in sorghum: 1) Control without inoculation, 2) Treatment of Seeds, 3) Application in

Furrow, 4) Application on cover after sowing and; 5) Application 10 days after emergence.

For the implementation of the experiment, samples of soil used for the installation in 5-liter pots were collected, classified as Dystrophic Red Latosol (Table 1).

**Table 1.** Soil analysis of the experimental campus of the Evangelical Faculty of Goianésia.

PH	P	K	Ca	MG	H+AL	MO
CaCl	Mgdm-3	Mmolc			g/kg-3	
5.9	3.4	0.108	1.7	1.01	2	21,5

To determine the growth and development, the following evaluations were made: stem thickness, root length, root volume, number of nodes, fresh shoot mass and aerial shoot dry mass, oven dried for 72 hours at 65 ° C, and plant height. The data received statistical treatment through the Assistat program (SILVA, 2016).

## RESULTS AND

The methods of inoculation were the following treatments: seed, furrow, cover, seedling and a treatment was the control. The stem thickness, root length, number of nodes, green mass, dry mass and root volume were evaluated in all treatments (Table 2).

**Table 2.** Results obtained from the methods of inoculation and control treatment in relation to stem thickness, root length and number of nodes.

Treatments	Thickness of the Stem	Length from Root	Amount of us	Green Mass	Dry mass	Root Volume
Seed	25,50a	62,50a	8,50a	139,25a	208,75a	37,50a
Groove	18,75a	46,50b	5,50b	88,50b	108,90b	56,25a
Roof	17,75a	46,00b	4,75b	32,25c	58,75c	15,00b
Seedling	17,50a	39,75b	4,50b	32,25c	69,90c	4,75b
Control	11,00b	29,75c	3,75b	25,00d	59,25c	56,25c
CV	11,04%	15,17%	32,61%	4,37%	7,42%	73,39%

Based on the results obtained, the stem thickness in relation to the inoculation of *Azospirillum* had a significant effect on the inoculated treatments, different from that found in the treatment of the control, which was the only difference from the others, showing that the inoculation of *Azospirillum* may have influence on the thickness of the stem. The same result was found in the corn crop by Dartora et. al. (2013), where the effect

of inoculation provided a larger basal diameter of the stem than the control.

The differences (stump thickness) of treatments from inoculations to control were statically higher, the same was also observed according to Revolti (2014) with inoculation in the maize crop for hybrid 2C707 PowerCore when inoculated in the sowing sowing stage V3 to V5 and the variety DSS-0404 when inoculated in the sowing groove V3 to V5 and in the seed + sowing

groove stage V3 to V5. In this same work, the commercial hybrid AG7098 PRO 2 and the experimental variety DSS-0402 do not presented significant values, demonstrating the importance of the *Azospirillum*-Plant interaction, as a factor that determines the good development and yield of the crop. Dartora et al. (2013) also observed the superiority of the inoculation in relation to the control in the corn crop with other genotypes. Increases in stem thickness may be related to the production of phytohormones by bacteria (RADWAN et al., 2004; MOREIRA et al., 2010). The thickness of the stem is highly related to the productivity, since it can accumulate more photoassimilates and consequently, greater production (KAPPES et al., 2011).

## DISCUSSION

But according to Kaneko (2013), its results indicated that it promoted a lower stem diameter, which was contrary to what was imagined from the inoculation with *Azospirillum*, the same reason, due to the absence of nitrogen fertilization.

The results obtained in this work to be explained by Dartora (2013), can be linked to the production of plant hormones by bacteria, such as cytokinins, gibberellins and auxins in treatments submitted to inoculation. But according to Bossolani et. al (2014), in a work with sorghum obtained in the treatments in the presence of inoculation obtained plants with smaller diameter of the stem.

Revolti (2014) observed a statistical difference in relation to stem diameter in maize when the author evaluated inoculation in the sowing groove, sowing groove in the V3 stage to the V5, in the seed and in the seed inoculation + sowing groove in the V3 stage to the V5. Plant density is the limiting factor for stalk thickness (STRIEDER et al., 2007). Density stimulates sowing due to competition between plants for light and

consequently reduces stalk thickness (SANGOI et al., 2002).

About the root length, treatment with inoculated seed had a significant effect, in relation to the other treatments inoculated. But the control treatment remained inferior to the other treatments. According to Didonet (1993) and Mello (2012), both studies with wheat evaluated root length and stated that the inoculation of wheat seeds with bacteria of the genus *Azospirillum* promoted root growth, further explains that these results can be attributed to plant hormones that interfere with growth, such as auxins.

The morphology of the root system can be modified by the *Azospirillum* bacterium because of the production of growth promoting hormones, which increase the number of radicles and the diameter of the lateral and adventitious roots (VVellet et al., 2000; VORPAGEL, 2010; MELLO, 2012).

In the number of nodes results, only inoculated seed treatment had a positive effect, showing a much larger number of nodes than the others, which remained relatively similar (Graph 3). The explanation given was the same, in relation to root length and stem thickness, this occurred due to the production of substances that promote growth by the bacterium (CAVALLET et al, 2000; VORPAGEL, 2010; MELLO, 2012; DARTORA, 2013).

Brzezinski et al. (2014), in their work with corn, using seed inoculation, observed that the length of the root of wheat seedlings of the cultivar BRS Tangará were influenced by the inoculation, being superior to the control. This is associated with increased capacity for root absorption and development in addition to stimulating the production of hormonal substances bringing balance to the plant (SANTOS & VIEIRA, 2005).

In the green mass, treatment with seed inoculated with *Azospirillum* bacterium had a very significant value in relation to the other treatments.

Furrow, cover and seedling treatments differed from control treatments, indicating that inoculation influences green mass production. This Result is different from that found by Bossolani et. al (2014). In their results, the total green matter with the presence and absence of inoculation was statistically similar, yet it complements that the green mass production could have been harmed by the tillering.

In the results of the dry mass quantity, in treatment with seed inoculated with nitrogen fixing bacteria there was a significant effect, in treatment with furrow with inoculation it maybe had a significant effect, less inferior to the one of the seed, but was superior to the treatments with inoculation by cover and seedlings and control treatments, they were similar in the results (Graph 5). Brzezinski et al. (2014), in their work with wheat, using inoculation via seed, observed that the length of the aerial part of wheat was influenced by the inoculation, being superior to the control.

This result is different from that found by Bossolani et. al (2014), who also about the dry mass, verified that the results with inoculated treatments and control treatment was statistically similar. According to Vorpagel (2010), its conclusion may explain the result obtained in the dry matter quantity of this work, where it was evidenced that the inoculation of the seeds in the corn crop with the bacteria *Azospirillum* brasilense is responsible for the increase of the dry matter accumulation rate, which may be related to increased nitrogen assimilation and the activity of photosynthetic enzymes.

In the work of Muller et al. (2016) observed that the highest foliar index (green mass) was used to inoculate *A. brasilense* via seed, in addition, there was also an increase in productivity in the crop. The increase in productivity and leaf mass may be related to the ability of the *Azospirillum* bacterium to fix nitrogen from the atmosphere (DOBBELAERE et al., 2003) and the production of plant hormones (OKON &

VANDERLEYDEN, 1997) acting on the root and functioning of the plant (PERIN et al., 2003).

In the root volume results, the control treatment also had a significant and statistically similar effect to treatments inoculated in furrow and seed. Being superior to inoculated treatment in cover and seedling. The result did not coincide with what was said by Vorpagel (2010), according to whom the gain with *Azospirillum* spp. is not only restricted to the N supply by biological nitrogen fixation (BNF), but also to the increase of the root surface of the plant and, consequently, to the increase of the soil volume.

## CONCLUSIONS

The inoculation with *Azospirillum* promoted positive effects on shoot thickness and root length, obtaining significant amounts.

The inoculated treatment with seed was the one that had better results and shows that it is the best alternative, for inoculation of *Azospirillum*.

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