Journal of Advances in Biology & Biotechnology

Volume 27, Issue 5, Page 1-12, 2024; Article no.JABB.114058
ISSN: 2394-1081

Azospirilla and Nitrogen Fertilization to Promote Maize Seed Development and Yield

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Authors' contributions

This work was carried out in collaboration among all authors. Author MTGDP conducted the experimental maintenance and data assessment. Author MHRF conducted the experimental supervision and intellectual contribution. Author RCDO conducted the experimental supervision and intellectual contribution. Author RMQL intellectual contribution. Author BW intellectual contribution. Author EML manuscript drift and critical review. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2024/v27i5757

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114058

Original Research Article

Received: 08/01/2024
Accepted: 13/03/2024
Published: 26/03/2024

ABSTRACT

Aims: This study evaluated the interaction of *Azospirillum brasilense* (Ab) strains and nitrogen fertilizer doses on maize.

Study Design: Laboratory experiment evaluated Ab strains (PCR and PTF) on maize seed germination and vigor (completely randomized design). Field experiment evaluated Ab strains and nitrogen fertilization on maize yield parameters (randomized block design).

Place and Duration of Study: The germination and vigor experiment (10 days in April 2019) was implemented in a controlled environment at the Seed Analysis Laboratory of the Universidade

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Federal de Uberlândia, Brazil. The field experiment (137 days in winter 2019) was implemented at the Fisioplant Research Station (Uberlândia, Brazil) under commercial maize crop management.

Methodology: In controlled conditions (laboratory), 200 seeds of maize (K9105VIP3, Riber KWS Seeds) were incubated (25 °C) with the Ab strain solutions for germination and vigor evaluations. In the field, 60,000 plants ha^{-1} (0.5 m space between sowing lines) of maize were cropped under combinations of nitrogen fertilizer and volumes of Ab strains (treatments) for leaf nitrogen, SPAD index, leaf count, fresh and dry maize shoot biomass, one-thousand grains weight, grain rows per maize ear, productivity (kg ha^{-1}).

Results: Treatment with 100 mL ha^{-1} of Ab PFT strain increased maize seed germination and vigor more than Ab PCR strain and control (no-inoculation or fertilizer). The number of grain rows of the 100% N + 150 mL ha^{-1} of Ab PFT strain and the maize shoot dry mass of the 100% N (no-inoculation) were higher than the control. The treatments did not affect the SPAD index, weight of one thousand grains, fresh maize shoot biomass, or productivity.

Conclusion: Using Ab with nitrogen fertilizer is a promising strategy to improve maize crop responses; however, more studies are needed to detail the ideal management of Ab strains for maize crops and yield construction.

Keywords: Zea mays L.; diazotrophic bacteria; biological nitrogen fixation; urea; maize grain productivity; maize crop; brazilian harvests; nitrogen; absorption.

1. INTRODUCTION

Maize (*Zea mays* L.) is crucial for integrating animal feed and human consumption. Brazil can grow three maize harvests per year. The first harvest comprises the sowing from October to December; the second is sown between January and March, and the third is between June and August. The third harvest is recent and has been expanding its importance. It results from short-cycle cultivars, irrigated areas, and sowing in milder winters, as in most Brazilian cerrado (Savanna-like biome) [1].

The average maize crop yield in Brazil is about 5.56 t ha^{-1} [2], a low average compared to the potential productivity of other countries, such as the United States (10.9 t ha^{-1}) [3]. However, the total production of the three Brazilian harvests, associated with high farming technology and intense land use, can reach up to three times the average maize crop yield.

Maize development depends on several factors, such as the efficiency of nitrogen (N) absorption and its translocation to the grains; nutrient dynamics is important since it is the most required nutrient by the crop [4]. The urea fertilizer is the most used due to its high N concentration and low cost-benefit compared to other nitrogen sources [5]. However, urea can lose N by volatilization and leaching, and research is needed to develop alternatives that improve N fertilization [6].

In this sense, public and private research, with the support of crop producers, seeks to identify strategies that can meet the nutritional demand for maize associated with low production costs and low environmental impact. These pursue to meet sustainable production principles and ensure food security for the human population. Using plant growth-promoting bacteria (PGPB) to fix atmospheric N_2 is a promising alternative to increase plant nutrition efficiency and decrease production costs [7].

The bacteria of the *Azospirillum* genus are PGPB in grass plants such as wheat, maize, and rice [8]. Seed inoculations, including *Azospirillum* spp., are routinely performed in maize, primarily through liquid formulations [9]. The literature review indicated that the *Azospirillum* spp. is associated with biological nitrogen fixation, phosphate solubilization, and plant protection against abiotic stress [10,11].

The present study evaluated the interaction of *Azospirillum brasilense* strains associated with urea doses on maize plant parameters and yield factors on maize.

2. MATERIALS AND METHODS

2.1 Seed Germination and Vigor Test

The germination experiment was installed in a controlled environment on April 24th, 2019, in the Seed Analysis Laboratory of the Federal University of Uberlândia facilities. The experiment comprised four treatments (control without seed treatment, two doses of *A. brasilense* 'PFT strain', and one dose of *A.*

brasilense 'PCR strain') and five replications, totaling twenty plots. The experimental parcels were distributed in blocks to avoid internal light interference in the incubator.

The test was done using germ papers soaked in 2.5 mL of distilled water for each gram of germ paper, two papers at the base, and one overlapping the seeds in each test roll. Each plot was structured with four rolls wrapped in germ paper containing 50 seeds each, totaling 200 maize seeds per plot, and stored in a fully translucent plastic bag inside the incubator at a constant temperature (25 °C). Distilled water was frequently added to each germ paper roll to maintain moisture. The treatments for the germination test in a controlled environment are described in Table 1.

The seed evaluations were done 4 and 7 days after the assembly and distribution of the germ rolls in the incubator, April 28th, 2019, and May 1st, 2019, respectively. As determined by the RAS [12], the seeds that generated normal seedlings at 4 and 7 days after the assembly of the germ rollers are indicators of vigor and germination, respectively.

Only normal seedlings with well-defined radicles and coleoptiles were considered in the count, and the other ones were discarded as dead seeds and/or abnormal seedlings. The treatment with the biological products considered the seed label information and the doses for 60,000 seeds. Seeds were treated and arranged to germinate soon after the seed inoculation. Seed samples (850 g) were weighed and treated with different product volumes (Table 2).

2.2 Field Experiment

The study was developed at the Fisioplant Research Statio, following the same conditions of a commercial field for maize crop cultivation in winter, irrigated whenever necessary by micro-perforated hoses of 58 m long. The rural property is located in Uberlândia, Brazil, close to the BR-050 road, km 83, at the geographic coordinates 18°59'48.4" S, 48°11'16.0" W and with an average altitude of 855 m.

According to Köppen's classification, the prevailing climate in the region is type Aw, with concentrated rains in summer and dry winter. According to the Brazilian Soil Classification System (SiBCS), the soil of the experimental area is classified as Red Latosol, with a medium texture and clay content of around 30%. The planting system included tillage, with shallow light harrowing and soil leveling for subsequent sowing.

The maize hybrid selected for the sowing of the experimental area was the K9105VIP3 of the Riber KWS Sementes Ltda industry. Seeds included industrial chemical treatment composed of thiamethoxan 35% at 120 mL per 60,000 seeds^{-1}, cyantraniliprole 80% at 40 mL per 60,000 seeds^{-1}, metalaxyl-m 2%, thiabendazole 15% and fludioxonil 2.5% at 30 mL per 60,000 seeds^{-1}, pirimiphos-methyl 50% at 7 mL 100 kg seed^{-1}, and deltamethrin 2.5% at 7 mL 100 kg seed^{-1}. The stand population was 60,000 seeds per hectare in the 0.5 m spacing between planting lines.

The experimental area was fertilized and plowed before sowing (April 20th, 2019). Fertilization was placed seven days before sowing, following a recommendation of 300 kg ha^{-1} of 08-28-16 (percentages of N, P_2O_5, and K_2O, respectively), buried in the planting groove. The sowing was manual (traction sowing), and the sowing depth was 3 cm above the buried fertilizer and 3 cm below the soil level.

Nine treatments were implemented: control without seed N fertilization or *A. brasilense*, four treatments including 50% of the recommended N dose associated with two doses of the *A. brasilense* 'PFT strain' and one dose of the *A. brasilense* 'PCR strain' formulated in a solution at the concentration of 5x10^9 CFU mL^{-1} and also without the microorganism, and four other treatments with 100% of the recommended N dose and the same associations with *A. brasilense*.

Four replications were performed for each treatment, totaling 36 plots of 8 sowing lines of 6 m length each, filling 24 m^2 of area per plot. The experiment was set as randomized block designs with nine treatments, each in the direction of the slope of the terrain. The treatments defined for the field research are in Table 3.

For N fertilization, the recommendation of 120 kg ha^{-1} of N was divided into two applications, half in the V4 phenological stage - the fourth leaf completely opened - and half in flowering. The fertilizer used as a source of N was urea (45% N) applied at the dose of 267 kg ha^{-1}. The first side-dressing fertilization was performed on May 6th, 2019, when the plants were in the V4 phenological stage. On July 10th, 2019, the second application was conducted at the beginning of the maize plant flowering. The T1 treatment was not fertilized, but in treatments, T2, T3, T4, and T5, 160 g of urea (equivalent to 66.75 kg ha^{-1}) were applied per plot. In treatments T6, T7, T8, and T9, 320 g of urea (equivalent to 133.5 kg ha^{-1}) were applied per plot, resulting in half the 100% recommended dose.

The maize seeds were treated with the *A. brasilense* PFT or PCR strains for the field experiment and considering the seed label information. Samples of 850 g of seeds were weighed and treated with the relative volumes of the products, according to Table 4. Seed samples were inoculated and sown soon after to minimize the harmful effects of the contact of *A. brasilense* with the chemicals of industrial seed treatment.

The field management of pests, diseases, and weeds was implemented using commercial standard products registered for each situation through crop monitoring. Whenever necessary, pesticides (according to the label's recommendation) and management tools were used, ensuring the sanity of the field.

2.3 Evaluations

2.3.1 Productivity

The productivity was evaluated by harvesting maize grains and correcting the harvested weight to 13% moisture. The experiment was harvested on September 4th, 2019, after 137 days of sowing. The four central lines were harvested in each plot, avoiding two lines at each edge. The harvested line was 5 m long, avoiding 0.5 m at each end of the line. The moisture correction followed the formula: $W_{final} = W_{initial} \times (100 – H_{harvest})$, where W_{final} is the corrected weight for moisture at 13%, $W_{initial}$ is the seed weight collected after harvest, and $H_{harvest}$ is the humidity of the sample collected.

After correcting the sample weight for standardized humidity, the weight of the sample collected in the useful area (10 m^2) was extrapolated to kg ha^{-1}, according to the formula $Y_{extrap.} = W_{plot} \times 1.000$, where $Y_{extrap.}$ is the productivity per hectare, and W_{plot} is the grain weight collected in the useful area.

2.3.2 Weight of thousand seed

The one-thousand grains weight was measured with sampling and repetitions of one-thousand grains after the harvest. After weighing four samples from each plot, the arithmetic mean was calculated and corrected for the standard humidity of 13%. The evaluation and calculation of the thousand seed weights were performed after the harvest of the plots.

2.3.3 Grain rows

Grain rows were estimated as the average number of rows present in 10 maize ears of each plot. The maize ears were collected randomly and within the useful area, discarding the surrounding areas. After counting, the arithmetic mean was performed, reaching the final data of each plot. The grain row count occurred before the September 1st, 2019 harvest.

2.3.4 Fresh and dry weight

The shoot fresh mass was evaluated on May 30th, 2019, 40 days after sowing. The shoot is considered the aerial part of the whole plant from the first internode above ground level, usually the basis for the sixth leaf of the maize plant. The same plants were used to calculate the dry weight after 26 days of drying in a greenhouse with constant temperature.

2.3.5 Foliar nitrogen

Leaf samples were taken from 10 leaves per plot, always choosing the opposite leaf and above the ear. After collection, they were dried until they stabilized the mass and ground in a Willey mill (2 mm), stored in paper bags, and taken to the laboratory for nutritional analysis of leaf N, according to the methodology suggested by Embrapa [13]. Samples were collected on July 8th, 2019, and the contents were measured on July 16th, 2019.

2.3.6 SPAD index

Three readings of the SPAD index were performed using the portable sPAD-502 meter (Soil Plant Analysis Development-502) at three crop moments: 48 days after sowing (DAS), 59 DAS, and 66 DAS, determining phenological stages V8, V10, and V12, where the plant was with the eighth, tenth and twelfth completely open leaf, respectively. The readings occurred on June 7th, 18th, and 25th, 2019, in sequence and were performed on the last leaf with the exposed sheath, determining the phenological stage of the plant. The number of leaves was also counted.

2.4 Statistical Analysis

The results of the laboratory tests and field experiment were submitted to the analysis of data residues (5% significance) using Action

Table 1. Treatments to evaluate maize germination test under controlled conditions

Treatments	Description
T1	100 mL ha^{-1} of *Azospirillum brasilense* 'PFT strain'
T2	150 mL ha^{-1} of *Azospirillum brasilense* 'PFT strain'
T3	100 mL ha^{-1} of *Azospirillum brasilense* 'PCR strain'
T4	0 mL ha^{-1} of *Azospirillum brasilense* (control)

Table 2. Treatment of 850 g of maize seeds for the germination test

Treatments	Description
T1	5.0 mL of *A. brasilense* 'PFT strain' + 2.5 mL distilled water
T2	7.5 mL of *A. brasilense* 'PFT strain'
T3	5.0 mL of *A. brasilense* 'PCR strain' + 2.5 mL distilled water
T4	7.5 mL of distilled water

Table 3. Defined treatments for field research

Treatments	Description
T1	0 of N + 0 of *A. brasilense* (control)
T2	50% of N + 0 of *A. brasilense* (control 50% of N)
T3	50% of N + 100 mL ha^{-1} of *A. brasilense* 'PFT strain'
T4	50% of N + 150 mL ha^{-1} of *A. brasilense* 'PFT strain'
T5	50% of N + 100 mL ha^{-1} of *A. brasilense* 'PCR strain'
T6	100% of N + 0 of *A. brasilense* (control 100% of N)
T7	100% of N + 100 mL ha^{-1} of *A. brasilense* 'PFT strain'
T8	100% of N + 150 mL ha^{-1} of *A. brasilense* 'PFT strain'
T9	100% of N + 100 mL ha^{-1} of *A. brasilense* 'PCR strain'

Table 4. Sample treatments of 850 g of seeds for the field experiment

Treatments	Description
T1	7.5 mL of distilled water
T2	7.5 mL of distilled water
T3	5 mL of *A. brasilense* ‘PFT strain’ + 2.5 mL of distilled water
T4	7.5 mL of *A. brasilense* ‘PFT strain’
T5	5 mL of *A. brasilense* ‘PCR strain’ + 2.5 mL of distilled water
T6	7.5 mL of distilled water
T7	5 mL of *A. brasilense* ‘PFT strain’ + 2.5 mL of distilled water
T8	7.5 mL of *A. brasilense* ‘PFT strain’
T9	5 mL of *A. brasilense* ‘PCR strain’ + 2.5 mL of distilled water

software in Microsoft® Excel and Tukey's test of average (5% significance) using SISVAR® software [14]. Correlations were performed on the means of each treatment to evaluate the interactions between the productivity components.

3. RESULTS AND DISCUSSION

3.1 Seed Germination and Vigor Test

No significant differences were detected for normal seedlings count (4 days after the assembly of the germination rollers) and for maize seed vigor (*A. brasilense* 'PFT strain' at 100 mL ha^{-1} and 150 mL ha^{-1}). Still, both doses presented higher vigor than the control (Table 5).

The 100 mL ha^{-1} (T1) dose presented higher vigor than the *A. brasilense* 'PCR strain' (T3). The sample treated with *A. brasilense* 'PCR strain' showed no difference between the control regarding the vigor of the seeds and the treatment with the *A. brasilense* 'PFT strain' at 150 mL ha^{-1} (T2). The increases observed for seed vigor treated with T1 and T2 relative to the control were 4.2% and 9.2%, respectively (Table 5).

The normal seedling count seven days after the assembly of the germination rollers indicated that the germination of the maize seeds treated with *A. brasilense* 'PFT strain' did not differ between the evaluated doses (100 and 150 mL ha^{-1}). However, the seeds treated with 100 mL ha^{-1} presented a germination index higher than the control and treatment with *A. brasilense* 'PCR strain'. *Azospirillum brasilense* 'PCR strain' was also similar to the control regarding seed germination.

Regarding the control, the 100 mL ha^{-1} dose of the 'PFT strain' (T1) performed better in germination and vigor, increasing by 9.6% and 9.2%, respectively. Therefore, the seed inoculation with *A. brasilense* improved biochemical attributes and possibly increased the N efficiency [15], which can be considered an economically and environmentally sustainable strategy for maize cultivation.

The germination and vigor improvements observed in the present study may also be related to stimuli in the root system development; Zambrano-Gavilanes et al. [16] observed such a situation. The improvements observed here could be due to the regulation of plant hormones (e.g. 3-indoleacetic acid - AIA), responsible for inducing the stretching and plant cell division [17] and the production of substances that promote plant growth [18].

3.2 Field Experiment

The number of leaves and SPAD indexes in three evaluation times (different maize phenological stages) was similar between the *A. brasilense* strains and the N doses evaluated (Table 6). The number of leaves ranged from 25 (T1) to 29 (T9), the SPAD index at 48 days after sowing (DAS) ranged from 37.7 to 42.8, while at 59 and 66 DAS, ranging from 37.5 to 42.4, and 41 to 47.5, respectively. These values are within the range of Alves et al. [18] and higher than those of Santos et al. [20].

The treatments were similar for the thousand grains weight (TGW), the productivity (kg ha^{-1}), and the shoot fresh mass of the maize plants (Table 7). The TGW ranged from 220.5 to 248.3 g, productivity ranged from 8.3 to 11.9 t ha^{-1}, and shoot fresh mass ranged from 1.1 to 1.4 kg plant^{-1}. The number of grain rows in the maize ear and the shoot dry mass presented differences between treatments. The treatment, including 150 mL ha^{-1} of *A. brasilense* 'PFT strain' and 100% of the N dose, stood out compared to the control (T1) for the number of grain rows in the maize ear.

The shoot dry mass of the treatment, including 100% of the N dose and no *A. brasilense* was superior to the treatment, including 50% of the N dose and no *A. brasilense* and the control (T1) (Table 7).

The yield components of each treatment were correlated at significant levels by Pearson's correlation index. Among the determinants of maize crop yield, the number of grain rows in the maize ear was directly correlated with the shoot fresh mass (89.55%) and with the shoot dry mass (74.95%) (Fig. 1).

The shoot fresh mass, besides presenting a significant correlation with the number of grain rows in the ear, also presented a significant correlation with the production (71.19%) and with the shoot dry weight (81.33%) (Figs. 2,3).

The third SPAD reading was done in the V12 maize phenological stage (66 DAS) and also significantly correlated with the first SPAD reading, done in V8 (48 DAS), and presented a correlation index of 71.92% (Fig. 3).

Similar to the present study, where no statistical difference was detected in maize grain productivity among the treatments, Libório et al. [21] reported that grain yield with the association of half of the N dose and *A. brasilense* was equivalent to the treatment that received the total N dose recommended for the crop. Such a result indicates the potential that *A. brasilense* has to reduce the use of mineral fertilizer in maize.

Gavilanes et al. [22] observed increased maize grain yield in two regions of Paraná state (Brazil), Londrina (4.4%) and Faxinal (11.9%), when *Azospirillum* was co-inoculated with *Anabaena cylindrica*. The combined inoculation of *Azospirillum* sp. and *Pseudomonas fluorescens* obtained the highest yield (19.70 t ha^{-1}) of fresh corn (about 15% bigger than the chemical control); such result indicates that the synergism of these two bacteria could be a sustainable strategy to improve yields and reduce the use of chemical fertilizers maize [23]. Barros et al. [24] also reported positive results of the *Azospuirillum* co-inoculation with *P. fluorescens* and *Bacillus subtilis* on maize plant height, colm diameter, and grains per ear.

Table 5. Average maize seedling vigor

Treatments	Vigor (%)	Germination (%)
T1	82.2 c	83.2 b
T2	77.2 bc	78.6 ab
T3	69.7 ab	74.1 a
T4 (control)	73.0 a	73.6 a
LSD	6.92	6.09

Similar averages in the column do not differ by Tukey's test (5% significance). LSD: least significant difference

Table 6. Averages of the number of maize leaves and SPAD indexes in three maize crop stages

Treatment	Leaves	SPAD 48 DAS	SPAD 2 59 DAS	SPAD 3 66 DAS
T1	24.85 a	38.07 a	38.98 a	40.95 a
T2	26.77 a	41.46 a	38.98 a	43.24 a
T3	28.00 a	37.68 a	37.45 a	41.52 a
T4	26.77 a	37.72 a	42.07 a	43.21 a
T5	25.55 a	40.09 a	40.35 a	45.72 a
T6	26.95 a	41.00 a	41.61 a	44.46 a
T7	27.65 a	42.58 a	42.37 a	47.46 a
T8	24.50 a	42.83 a	39.76 a	44.42 a
T9	29.05 a	41.86 a	42.73 a	44.26 a
LSD	7.38	6.16	9.27	8.46

Similar averages in the column do not differ by Tukey's test (5% significance). T1: Azospirillum brasilense (Ab) and urea absent; T2: Ab absent and 50% of urea dose; T3: 100 mL ha^{-1} of Ab 'PFT strain' and 50% of urea dose; T4: 150 mL ha^{-1} of Ab 'PFT strain' and 50% of urea dose; T5: 100 mL ha^{-1} of Ab 'PCR strain' and 50% of urea dose; T6: Ab absent and 100% of urea dose; T7: 100 mL ha^{-1} of Ab 'PFT strain' and 100% of urea dose; T8: 150 mL ha^{-1} of Ab 'PFT strain' and 100% of urea dose; T9: 100 mL ha^{-1} of Ab 'PCR strain' and 100% of urea dose. LSD: least significant difference

Table 7. Averages of a thousand-grain weight (TGW), productivity (kg ha^{-1}), number of grain rows, shoot fresh mass (SFM), and shoot dry mass (SDM)

Treatment	TGW g	Productivity kg ha^{-1}	Grain row	SFM g $plant^{-1}$	SDM g $plant^{-1}$
T1	220.50 a	8281.44 a	15.70 b	1192.50 a	277.50 b
T2	224.50 a	9456.55 a	16.00 ab	1210.00 a	277.75 b
T3	237.75 a	9445.24 a	16.55 ab	1297.50 a	329.00 ab
T4	238.50 a	11994.52 a	16.30 ab	1382.50 a	488.50 ab
T5	233.00 a	11894.71 a	16.30 ab	1337.50 a	364.50 ab
T6	230.00 a	10279.34 a	16.85 ab	1416.00 a	512.00 a
T7	248.25 a	10423.76 a	16.25 ab	1256.25 a	425.00 ab
T8	243.75 a	11900.69 a	17.15 a	1610.00 a	506.50 ab
T9	248.25 a	10445.32 a	16.42 ab	1292.50 a	343.75 ab
LSD	54.16	4793.78	1.30	698.68	229.66

Similar averages in the column do not differ by Tukey's test (5% significance). T1: Azospirillum brasilense (Ab) and urea absent; T2: Ab absent and 50% of urea dose; T3: 100 mL ha^{-1} of Ab 'PFT strain' and 50% of urea dose; T4: 150 mL ha^{-1} of Ab 'PFT strain' and 50% of urea dose; T5: 100 mL ha^{-1} of Ab 'PCR strain' and 50% of urea dose; T6: Ab absent and 100% of urea dose; T7: 100 mL ha^{-1} of Ab 'PFT strain' and 100% of urea dose; T8: 150 mL ha^{-1} of Ab 'PFT strain' and 100% of urea dose; T9: 100 mL ha^{-1} of Ab 'PCR strain' and 100% of urea dose. LSD: least significant difference

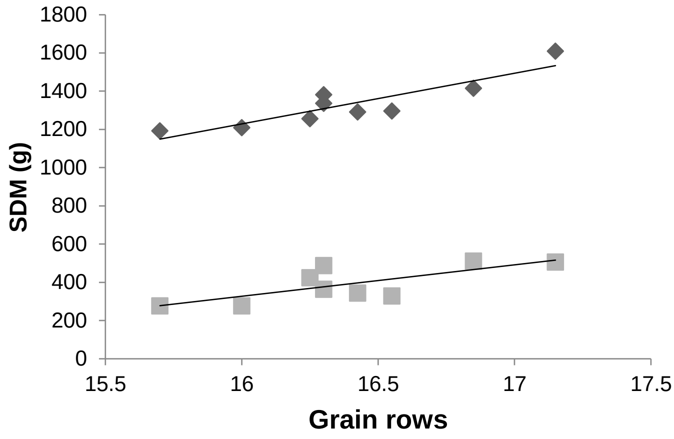

Fig. 1. Correlation graphics between grain rows and the shoot fresh mass (upper graphic) and shoot dry mass (lower graphic) in maize

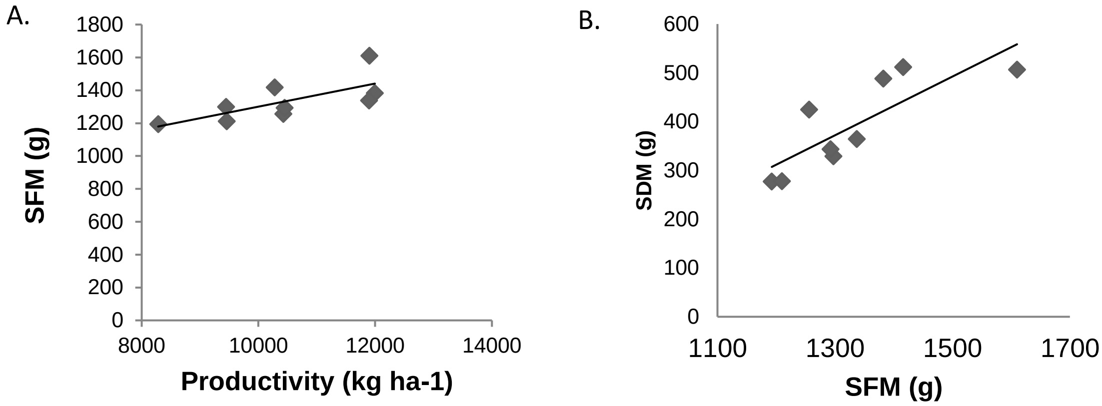

Fig. 2. Correlation graphic (A) between maize grain productivity and the shoot fresh mass (SFM), and (B) between SFM and shoot dry mass (SDM) in maize

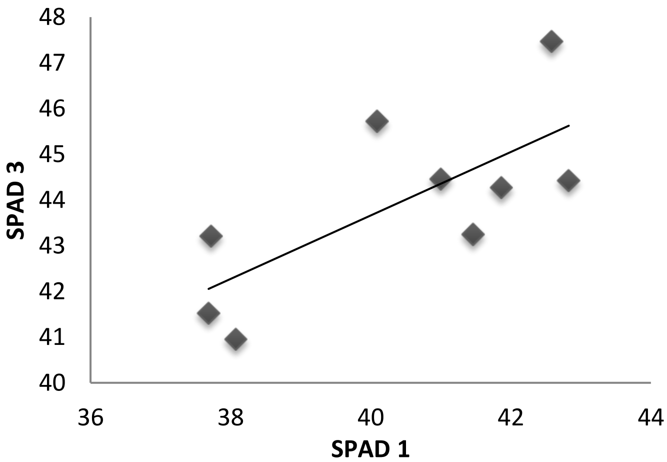

Fig. 3. Correlation graphic between SPAD 1 and 3 indexes

Kaneko et al. [25] studied different N doses (0, 45, 90, 135, and 180 kg ha^{-1}) with and without inoculation (*A. brasilense*) and observed higher maize yields at the higher doses tested but without inoculation. On the other hand, Pereira et al. [9] observed that the dose of 2 grams of inoculant per kilogram of seeds, associated with N fertilization (90 kg ha^{-1}), presented the highest crop yield. According to Garcia et al. [26], with the increased population of bacteria (8 g kg^{-1}), a mineral N intake greater than 90 kg ha^{-1} may be indispensable to achieve high crop yields.

Although extensive reports have found superiority in inoculating, there is also a lack of constant positive responses to production. Rockenbach [27] and Besen et al. [17] concluded that the inoculation technique including *A. brasilense* does not replace the use of N fertilizers or allows dose reductions, corroborating the present study, where the best performance was related to 100% of the applied N dose. On the other hand, Portugal et al. [28] observed that seed inoculation with *A. brasilense* generated a low final plant population, plant height, and mass of a thousand maize grains.

Barbosa et al. [29] performed a meta-analysis with 60 studies of maize inoculation with *Azospirillum* sp. comprising 103 field trials in 54 locations in Brazil. The authors reported that bacteria inoculation to maize increased 12.1% root mass, 4.3% N leaf concentration, 5.4% grain yield, and 3.6% N in grains. However, inoculation benefits were higher (+21%) at yields lower than 3000 kg ha^{-1} and at lower N rates (≤ 50 kg ha^{-1}, +8%). Such differences in response to maize inoculation can also be attributed to factors such as the number of viable cells per seed, the cell viability, the plant genotype, the *Azospirillum* genus, the inoculant physical placement, the biochemical characteristics and competition with other microorganisms native to the soil [30,31].

4. CONCLUSION

Inoculation with *Azospirillum brasilense* 'PFT strain' and 100 or 150 mL ha^{-1} concentration improved maize seed vigor. The 100 mL ha^{-1} concentration presented the best maize germination performance. The field treatment fertilized with 100% of the recommended nitrogen dose associated with 150 mL ha^{-1} of *Azospirillum brasilense* 'PFT strain' stands out regarding the number of grain rows per maize ear. The maize grain productivity revealed considerable numerical differences among the treatments (*Azospirillum* and N doses), especially between the control and the inoculated treatments. Still, the disparities did not reach statistical significance.

ACKNOWLEDGEMENTS

Agrarian Science Institute (ICIAG) of the Federal University of Uberlândia (UFU).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114058