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# MULTIFUNCTIONAL FLUORESCENT *Pseudomonas*: EFFECTS ON MAIZE DEVELOPMENT AND TOOLS FOR THEIR SELECTION

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**Abstract**
Maize is one of the most cultivated cereals worldwide. Despite the low nutrient availability in the soil, high amounts of fertilizers are applied causing economic and environmental impacts. Then, Plant Growth Promoting Rhizobacteria (PGPR) as Fluorescent Pseudomonas can be utilized as an alternative. The present work aims to analyze the effect of Pseudomonas isolates on maize development and production and verify the relationship between growth mechanisms and IAA production. Sixteen Pseudomonas isolates were tested in vitro to produce IAA, ACC deaminase, siderophores, and solubilize Fe and Al phosphates. Three isolates (CBSAL02, CBSAL05, and CBSAL06) were selected for the field experiment, in which an A. brasilense inoculant was the control, generating five treatments with four replications. More than 50% of the isolates demonstrated the tested mechanisms. Only CBSAL05 did not produce siderophore or could fix N. Inoculations with A. brasilense and Pseudomonas isolates increased leaf N content among the selected isolates. The CBSAL06 isolate increased productivity, thus demonstrating the potential use as an inoculant.

**Keywords:** AACDesaminase. Phosphates solubilization. Rhizobacteria. Siderophore. *Zea mays.*

## 1. Introduction

Maize (*Zea mays* L.) is one of the most cultivated cereals worldwide, and the second most cultivated grain in Brazil, after the soybean (Conab 2023). Brazilian grain production in the agricultural sector is significant to the country's economy because it produces food, raw materials and generates jobs.

The low availability of nutrients in the Brazilian soils is one of the main affecting plant growth (Barroso and Nahas 2005). High fertilizer doses are applied to solve this problem, which increases production costs (Rodrigues et al. 2015) since most of the raw material is imported (Fernandes et al. 2009).

The international fertilizer market has been limited by factors such as the COVID-19 pandemic, the energy crisis in China, the European countries, and the Russian-Ukraine war (Arndt et al. 2023). These countries supply fertilizer to Brazil.

High levels of exogenous fertilizers in the soil can also cause ecosystem imbalance, reduction of soil microbiota, and water contamination, thus highlighting the importance of searching for alternative practices to increase their uptake by plants or reduce their application (Lopes and Guilherme 2000). The

use of microorganisms to stimulate plant growth can minimize these effects. Some microbial mechanisms can be Biological Nitrogen Fixation (BNF), solubilization of nutrients such as P, and production or reduction of plant phytohormones (Nascimento et al. 2019; Balbinot et al. 2020; Keswani et al. 2020). Plant Growth Promoting Rhizobacteria (PGPR) inoculation increases nutrient uptake, reducing fertilizer use and environmental and economic costs (Ratz et al. 2017; Botelho and Brasil 2023).

Among PGPR, fluorescent *Pseudomonas* and *Bacillus* spp. have been studied and used to increase nutrient uptake since they can solubilize phosphates (Botelho et al. 2019) to produce auxins such as Indoleacetic Acid (IAA) (Keswani et al. 2020), responsible for stimulating root growth, and ACC deaminase production that degrades ethylene precursor (Zhang et al. 2018; Nascimento et al. 2019), which is produced at biotic or abiotic stresses. These bacteria are also important for bio-protection, inhibiting plant pathogens (Balbinot et al. 2020; WIN et al. 2022). Thus, bacterial isolates with different mechanisms can potentialize the stimulation for plant growth. This bacterial inoculation cannot replace yet agricultural inputs but can reduce them by maintaining or even improving plant production (Bernd et al. 2014; Mazzuco et al. 2023).

Several analyses and trials are performed on the bacterial isolates, and those holding the most relevant mechanism(s) are selected. However, improving this selection through technological tools can get more efficient results. Therefore, in this work, besides analyzing the effect of *Pseudomonas* isolates on maize growth and production, it was possible to evaluate differences among the isolates through cluster analysis regarding IAA production, and it was verified the relationship among other growth mechanisms analyzed and IAA production.

## 2. Material and Methods

### *In vitro* plant growth promotion mechanisms analyses

The fluorescent *Pseudomonas* were previously tested for IAA production, $Ca_3(PO_4)_2$ solubilization, and BNF (Botelho et al. 2019). Sixteen isolates were analyzed for Fe and Al phosphates solubilization, siderophore, and ACC deaminase productions. The isolates previously grew in flasks containing 5 mL of King B media (King et al. 1954) for 48 hours at 27° C to perform each analysis.

Media containing $FePO_4$ and $AlPO_4$, adjusting pH according to the methodology was used for Fe and Al phosphate solubilization (Nahas et al. 1994). Each medium received 50 µL of each isolate at equidistant points. It was observed the solubilization halos after 72 hours at 27° C.

For ACCD (1-aminocyclopropane-1-carboxilate deaminase) production, 50 µL of each isolate isolates grew on specific media plates containing ACC as a unique N and C source (Gupta and Pandey 2019; Glick and Nascimento 2021), at equidistant points. After 72 hours at 27° C, the growth of isolates was observed indicating ACCD production.

The isolates grew on plates containing a specific medium to evaluate siderophore production (Schwyn and Neilands 1987), in which 50 µL of each isolate was placed at equidistant points. After incubation for 72 h at 27° C, the presence of a blue-green halo indicated the siderophore production.

### Efficiency analysis of fluorescent *Pseudomonas* spp isolates in maize.

Three isolates (CBSAL02, CBSAL05, and CBSAL06) were selected for field evaluation to check their performance in inoculation at corn seeds. The experiment was carried out at the Experimental farm of Universidade Federal de Santa Catarina – *campus* Curitibanos (Brazil) in the 2022/2023 harvest. The area is located at geographic coordinates 27º16'26.55" S and 50º30'14.41" W and an altitude of approximately 1000 meters. The regional climate is classified, according to Köppen-Geiger, as temperate Cfb – humid mesothermal and mild summer. The soil is classified as Haplic Cambisol with Clay Texture with wavy relief (Santos et al. 2018). Table 1 shows the soil analysis in which the experiments were carried out.

The liming was not necessary according to soil analyses. The fertilizing was performed based on SBCS (2004). At sowing, it was applied 31.5 kg of N. $ha^{-1}$ (68.5kg urea), corresponding to 50% of N total

fertilization; 115.5 kg de $P_2O_5$. $ha^{-1}$ (280.7 kg of triple superphosphate) and 42 kg de $K_2O$. $ha^{-1}$ (70 kg of KCl). Thirty days after sowing (DAS), fertilizing top dressing was carried out with 50% N remaining.

**Table 1.** Soil analyses of the experimental area.

| Soil characteristics | 2021/2022 |
|---|---|
| pH $CaCl_2$ | 5.50 |
| P ($mg.dm^{-3}$) | 23.52 |
| K ($mg.dm^{-3}$) | 70.20 |
| Ca ($cmolc.dm^{-3}$) | 10.48 |
| Mg ($cmolc.dm^{-3}$) | 4.95 |
| Al ($cmolc.dm^{-3}$) | 0.00 |
| H + Al ($cmolc.dm^{-3}$) | 4.28 |
| MO ($g.dm^{-3}$) | 37.84 |
| SB ($cmolc.dm^{-3}$) | 15.61 |
| CTC pH7 (cmolc.dm-3) | 19.89 |
| Zn ($mg.dm^{-3}$) | 3.20 |
| Fe ($mg.dm^{-3}$) | 22.20 |
| V (%) | 78.48 |
| Mn ($mg.dm^{-3}$) | 17.90 |
| Cu ($mg.dm^{-3}$) | 3.80 |
| Sampling Depth: 20cm | |

The fluorescent *Pseudomonas* isolates CBSAL02, CBSAL05, and CBSAL06 were inoculated in tubes containing 10 mL of King B liquid medium (King et al. 1954) and incubated for 24h at 27º C to prepare the inoculants. After this period, each isolate was inoculated in flasks containing one (01) g of peat sterilized for 72 hours at 27° C. The control received 1 g of peat without inoculation and kept under the conditions described. The isolates' inoculant production calculations were based on the recommendation of 100 g of peat inoculant for 50 kg of seeds, plus 300 mL of 10% sugar solution. The commercial inoculant treatment containing the Abv-5 and Abv-6 strains of *A. brasilense* followed the manufacturer's dosage recommendation.

The plots had 3 m x 4 m, totalizing 12 $m^2$, with eight sowing lines, and 6 $m^2$ of useful area. The spacing was 50 cm between rows and 30 cm between plants, adding up to 1.600 plants. The experimental design was randomized blocks, with five treatments and four replications with 20 plots. The treatments were: CBSAL02 isolate inoculation, CBSAL05 isolate inoculation, CBSAL06 isolate inoculation, control without inoculation, and commercial inoculant containing Abv-5 and Abv-6 strains of *A. brasilense*.

The growth parameters evaluated were plant height, ear insertion, stem diameter, and leaf N content at the R1 phenological stage of flowering. Five plants from the useful area of each plot were evaluated. The plant height was measured from its base to the last leaf. The ear insertion was measured from the plant base to the ear insertion base. Both measurements were taken with tape. The stem diameter was taken with a digital caliper, at the second node above the ground. To analyze leaf N content, the first leaf below the ear was removed from each plant. The central third of the leaf was removed, stored in a paper bag, and dried at 45° C for 48 h. Subsequently, the leaves were crushed and taken for analysis by steam distillation and titration (Tedesco et al. 1995).

At the harvest, five plants were collected from the useful area of each plot to evaluate the number of rows per ear (NRE) and number of grains per row (NGR). The ears were threshed for productivity analysis. The grain mass was weighed, and a sample was taken to measure moisture. Productivity was calculated in $kg.ha^{-1}$ after correcting humidity to 14%.

The data were submitted for analysis of variance by using the F test with 5% significance, and when significant the means were compared by the Tukey test at 5% significance, by Sisvar program version 5.8 (Build 92) (Ferreira 2014).

**Statistical analysis for selection of multifunctional rhizobacteria**

Fluorescent *Pseudomonas* isolates were ordered according to their IAA production potential using Euclidean distance and grouped by Ward's k-means hierarchical method. The cluster analysis was

computed using the *factoextra* package (Kassambara and Mundt 2020), in the statistical software R (R core team 2021).

Using analysis of variance (ANOVA), the significant differences ($p < 0.05$) in IAA production were tested for mechanisms such as Biological Nitrogen Fixation (BNF), ACCD production, siderophore production, and solubilization of Al, Fe, and Ca phosphates. For this, *Pseudomonas* isolates were grouped into two classes related to the occurrence or not of the tested mechanisms, considering them positive or negative. ANOVA was applied to test differences in IAA production between classes for all mechanisms evaluated. The Shapiro-Wilk normality test and Levene's test of homogeneity of variances were used to verify the statistical assumptions of the ANOVA that was computed using the car package (Fox and Weisberg 2019), in the statistical software R (R core team 2021).

## 3. Results

### Growth-promoting mechanisms of fluorescent *Pseudomonas* isolates *in vitro* analyses.

Ethylene is a plant hormone linked to several physiological aspects, such as fruit ripening and senescence (Taiz et al. 2017; Iqbal and Khan 2017). Plants exposed to biotic or abiotic stresses have ethylene levels increased, reducing their growth and productivity, and if not controlled, it can cause their death (Gupta and Pandey 2019). Several Rhizobacteria are capable of degrading ACC (1-aminocyclopropane-1-carboxylate), a precursor of ethylene exuded by the roots, through the ACC deaminase enzyme that hydrolyzes this substance, reducing ethylene levels in the plant and the effects of stress (Gamalero and Glick 2015; Gupta and Pandey 2019). Rhizobacteria ACCD-producing are common, especially in soils with limiting conditions (Gamalero and Glick 2015). One of the first microorganisms described as synthesizers of ACC deaminase was a strain of *Pseudomonas* sp. (Honma and Shimomura 1978). In this work, 14 fluorescent *Pseudomonas* isolates could degrade ACC *in vitro*, suggesting relevant occurrences in this community (Table 2). Fluorescent *Pseudomonas* are typically growth promoters, and the absence of ACCD production considerably decreased the ability to promote root elongation in species such as canola and maize (Glick and Nascimento 2021). It demonstrates the relevant number of ACCD-producing isolates obtained is coherent since fluorescent *Pseudomonas* belong to a bacterial group, which is common in the rhizosphere of several plants, and it is involved in many plant growth and development processes, such as reducing ethylene levels by degradation of ACC (Glick and Nascimento 2021).

Among the tested mechanisms in this work, the siderophores production had the lowest percentage of isolates (56.25%) (Table 2). However, more than half of the collection showed this ability, suggesting the relevance of this mechanism. Fe is an essential element for all living beings. In agricultural soils, its availability is reduced due to increased pH (Lemanceau et al. 2009), limiting its absorption by plants and microorganisms that have developed strategies to capture Fe from the environment. Some microorganisms release Fe-chelating compounds, called siderophores, which are absorbed and remain in the cytoplasm (Hider and Kong 2010). The main siderophore produced by *Pseudomonas* is pyoverdines (Lemanceau et al. 2009; Rehm et al. 2023). Through this mechanism, rhizobacteria such as *Pseudomonas* can help plant growth directly, influencing Fe nutrition (Lurthy et al. 2020) or indirectly, removing Fe from the environment and reducing phytopathogen communities (Kumar et al. 2018). The isolate CBSAL02, which could produce a siderophore, showed lethal action against the nematodes *Meloidogyne javanica* and *Ditylenchus* spp. eggs (Turatto et al. 2018).

Fourteen isolates (87.5%) solubilized $FePO_4$, and ten ones (62.5%) solubilized $AlPO_4$ (Table 2). The existence of a significant community of phosphate-solubilizing bacteria close to the roots has been described by several authors (Elhaissoufi et al. 2020), following the results of the present work. In previous work, isolate CBSAL02, when associated with isolate EB17 of *Bacillus* spp., significantly increased garlic productivity, even without phosphate fertilization, indicating their ability to solubilize existing P in the soil (Mazzuco et al. 2023). The P availability in soils is low due to its ions' high reactivity and retention associated with soil constituents (Mendes and Reis Junior 2003; Barbosa et al. 2022). The organic P can be immobilized in organic matter, and inorganic P can precipitate with Al, Fe, and Ca, and can be adsorbed to Fe and Al oxides in clays or compounds of lower reactivity (Barbosa et al. 2022). In weathered soils, such as

the Brazilian ones, the retention of P ions in Fe and Al oxides or precipitates can significantly reduce their availability (Mendes and Reis Junior 2003; Barbosa et al. 2022). Phosphate fertilizers are used to provide adequate P amounts to crops. However, only 20% of the quantity applied is absorbed by plants, and the major part is absorbed by colloids or forms poorly soluble compounds. (Mendes and Reis Junior 2003). Due to its ability to solubilize phosphates, the soil microbiota, especially rhizobacteria, has been intensely studied (Mukhtar et al. 2020). *Pseudomonas* from the fluorescent group demonstrated significant effects on the growth and production of several plant species due to their ability to solubilize phosphates (Bilal et al. 2021; Mazzuco et al. 2023).

**Table 2**. *In vitro* plant growth promotion mechanisms of Pseudomonas isolates from the rhizosphere of garlic cultivated in the Santa Catarina State Plateau.

| Isolates | ACCD | Siderophore | $FePO_4$ | $AlPO_4$ |
|---|---|---|---|---|
| CBSAL02 | + | + | + | + |
| CBSAL03 | + | + | + | - |
| CBSAL04 | + | + | + | - |
| CBSAL05 | + | - | + | + |
| CBSAL06 | + | + | + | + |
| CBSAL07 | - | - | - | + |
| CBSAL08 | + | - | + | + |
| CBSAL09 | + | + | + | - |
| CBSAL10 | + | - | + | + |
| CBSAL11 | + | + | + | - |
| CBSAL13 | + | + | + | + |
| CBSAL14 | + | - | + | - |
| CBSAL16 | + | + | + | + |
| CBSAL19 | + | - | + | + |
| CBSAL22 | - | + | - | + |
| CBSAL23 | + | - | + | - |

(+) - positive
( –) - negative

**Efficiency of fluorescent *Pseudomonas* spp. isolates inoculation in maize**

Three isolates were selected for field trials. Parameters such as plant height and ear insertion are important, as they are directly linked to the plant's resistance to lodging and leaf area for photosynthetic activity. However, they were not influenced by the inoculation of these bacteria (Table 3). Similar results were obtained by Zucarelli et al. (2011) in maize inoculated with *Pseudomonas fluorescens* isolates. The inoculation did not show statistical differences concerning ear height and insertion. Similar results were obt ained by Oliveira et al. (2012) regarding this parameter in response to *Pseudomonas* inoculation in maize, too. The inoculation of *Azospirillum brasilense* and different levels use of N in maize did not result in differences in plant height (Repke et al. 2013). Similar results were also observed by Dos Santos et al. (2022) in maize inoculated with *A*. *brasilense*.

The isolate CBSAL06 resulted in a stalk diameter average statistically equal to the control and the AB inoculation and higher than the CBSAL02 and CBSAL05 inoculations. The parameter is linked to lodging tolerance, which can cause vascular interruption of the stalk, causing difficulties in the growth and development of the plant, reflecting on grain yield and quality (Gomes et al. 2010). Lodging can be influenced by plant height and stalk diameter since a larger stalk diameter can increase the capacity to withstand plant breakage or tipping over (Sangoi et al. 2002). In this sense, the CBSAL06 inoculation could increase resistance to lodging because it reached the highest average diameter, even without differing statistically from the control and AB inoculation. In addition, the stalk supports the leaves and flowers and serves as a photo assimilates reserve, reflecting grain yield and quality (Gomes et al. 2010). In a study

carried out with inoculation of *Azospirillum brasilense* and *Herbaspirillum seropedicae* in maize, Dartora et al. (2013) observed a 15% increase in stalk development compared to the control, in vegetative and reproductive stages that was attributed to biological nitrogen fixation (BNF) and the IAA production.

Part of the N absorbed by plants is transferred to the ears and, consequently, to the grains. So, parameters such as NRE (Number of Rows per Ear) and NGR are significant in evaluating the effect of growth promoters on plant production. For NRE, all treatments were statistically equal, except the CBSAL05 inoculation treatment, which showed a lower average (Table 3). The lack of BNF by CBSAL05 may have influenced this reduction, but further studies are necessary to deepen knowledge. Similar results to NRE, in which inoculated maize with *Pseudomonas fluorescens* showed no difference regarding the control, were reported by Zucareli et al. (2011). However, to correlate it to yield, other parameter analyses are recommended (Olivoto et al. 2018) since an increase in NRE can signify an increase in the number of grains. Nevertheless, they may have low specific mass (Lopes et al. 2007) and reflect negatively on yield.

The NGR is related to the ear length and the interaction between genotypes and environments (Olivoto et al. 2018). An increase in NGR can mean a bigger number of grains and, consequently, a greater plant yield. The *A. brasilense* and CBSAL06 inoculations reached the highest NGR averages compared to the control, indicating they could stimulate the ears' size, which can reflect on plants' production. Guimarães and Klein (2023) obtained similar results when analyzing phosphate fertilizer doses to maize associated with *P. fluorescens* inoculation. They observed NGR differences among treatments with different phosphate fertilizer doses associated or not with bacterial inoculation. However, they do not observe the effect on NRE.

The leaf N content determines the elements' amount absorbed by the roots and transported to the shoot and, consequently, to the grains by nitrogen fertilization or BNF. The content considered suitable for maize is around 27.0 to 35.0 g $kg^{-1}$ of N in leaf tissue (sufficiency range) (Raij et al. 1997; De Oliveira et al. 2022). The N foliar increase of AB, CBS02, and CBS06 could be related to BNF since CBSAL05 did not show this mechanism *in vitro* (Botelho et al. 2019) and all *Pseudomonas* isolates showed the ability to produce IAA (Botelho et al. 2019).

IAA production is a relevant mechanism promoting N uptake. Auxins, such as IAA, are involved in root elongation (Taiz et al. 2017), and their increase, respecting hormonal balance, can stimulate root growth and, consequently, the absorption of water and nutrients. It is important to stress the increase in leaf N content provides photosynthesis increase since around 50% to 70% of the total N is related to enzymes present in chloroplasts (Booij et al. 2000).

For yield purposes, it is significant to notice the total fertilization for the crop was applied to the experiment, indicating the isolate CBSAL06 enhanced its use, resulting in a productivity gain increase. It is important to highlight the *A. brasilense* product formulation has two strains (Abv-5 and Abv-6), while the *Pseudomonas* isolates were inoculated individually. Although there was no statistical difference observed among CBSAL02, CBSAL05, *A. brasilense* inoculations, and the control, the increase provided by these bacteria (16.2%, 13.3%, and 18.0%, respectively) encourages further studies with these *Pseudomonas* isolates, may be combined to test their interactions.

The maize productivity average for Santa Catarina State in the 2022/23 harvest was 8.16 $kg.ha^{-1}$, according to Conab (2023), indicating that the bacterial inoculations were effective and obtained higher values. Only the control had a lower yield average (7.40 $kg.ha^{-1}$). Rhizobacteria such as *Pseudomonas* spp. have increased maize productivity, even when N fertilization is reduced. Jiang et al. (2022) observed, in a field experiment, that maize yield increased by 5.6% and 5.9% in the absence and presence of exogenous nitrogen fertilizer, respectively, due to an isolate of *Pseudomonas stutzeri* inoculation. These results reinforce those obtained with the CBSAL06 isolate, suggesting this rhizobacterium has the potential as an inoculant with the possibility of reducing fertilizers, making cultivation more sustainable.

Among the isolates tested in the field experiment (CBSAL02, CBSAL05, and CBSAL06), all solubilized Fe phosphate produced ACCD (Table 2). Isolates CBSAL02 and CBSAL06 showed N *in vitro* fixation capacity, except for CBSAL05. These isolates' inoculation increased the leaf N content compared to the control (Table 3). However, even with no statistical difference, the CBSAL05 presented the lowest average for this parameter. The isolate CBSAL06 reached the highest maize yield average (Table 3) and differed from the control. This isolate produced the lowest amount of IAA (15.25 µg. $mL^{-1}$ - Figure 1). These results suggested

that several mechanisms are interrelated for the effect of isolates inoculation on maize plants, especially the production of IAA.

**Table 3.** Effect of fluorescent *Pseudomonas* isolates of inoculation on maize growth and development.

| Treatments | Plant height (cm) | Ear insertion (cm) | Stalk diameter (mm) | Leaf N content [1] | NRE | NGR | Yield (t.ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Control | 2.66 | 1.41 | 25.65$^{ab}$ | 22.13$^{b}$ | 18.80$^{ab}$ | 34.55$^{c}$ | 7.40$^{b}$ |
| CBSAL02 | 2.77 | 1.43 | 24.35$^{b}$ | 29.47$^{a}$ | 18.90$^{ab}$ | 35.30$^{bc}$ | 8.83$^{ab}$ |
| CBSAL05 | 2.72 | 1.41 | 23.90$^{b}$ | 27.86$^{a}$ | 18.25$^{b}$ | 35.40$^{bc}$ | 8.54$^{ab}$ |
| CBSAL06 | 2.74 | 1.51 | 28.40$^{a}$ | 29.41$^{a}$ | 19.20$^{ab}$ | 36.75$^{b}$ | 9.16$^{a}$ |
| AB | 2.80 | 1.44 | 26.75$^{ab}$ | 29.49$^{a}$ | 19.40$^{a}$ | 38.85$^{a}$ | 9.03$^{ab}$ |
| Average | 2.74 | 1.44 | 25.81 | 27.67 | 18.91 | 36.17 | 8.60 |
| ANOVA | ns | ns | * | * | * | * | * |
| V.C. (%) | 5.85 | 8.68 | 16.36 | 6.02 | 6.16 | 6.56 | 23.07 |

*Averages followed by the same letter do not differ statistically by Tukey test at 5% significance level.
AB – *A. brasilense* inoculation
ns – not significant
[1] gN.kg$^{-1}$dry matter (DM.)
NRE - number of rows per ear
NGR - number of grains per row

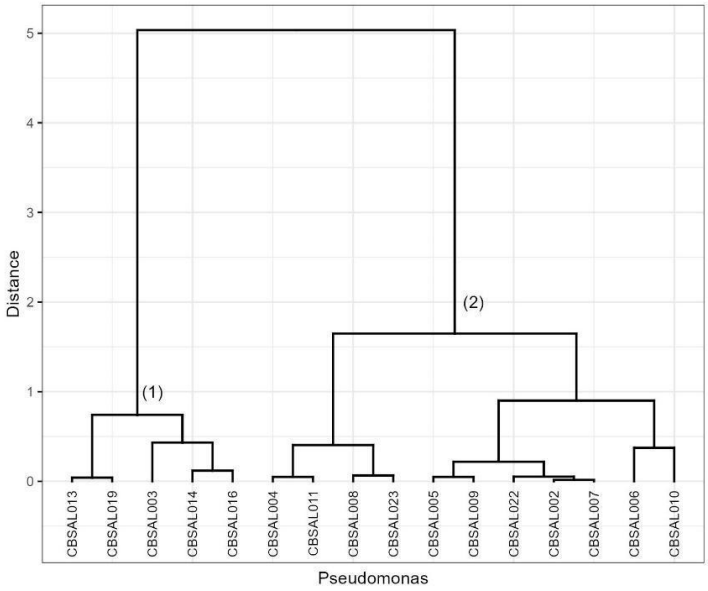


**Figure 1.** Hierarchical clustering of fluorescent Pseudomonas spp. by IAA production.

**Selection of multifunctional Rhizobacteria**

In the present work, the isolate CBSAL06 produced a lower amount of IAA (15.25 µg. mL$^{-1}$ - Figure 1), suggesting it can be one parameter for selecting rhizobacteria with more than one effective mechanism for plant growth. High concentrations of auxins can alter the hormonal balance in the plant, acting as an inhibitor of its growth (Taiz et al. 2017), interfering with other mechanisms of action, and preventing interaction between plants and bacteria.

The relationship between IAA production and the mechanisms tested showed significance among BNF, Fe phosphate solubilization, and ACCD production (Figure 2). The ability to fix N, solubilize Fe phosphate, and produce the ACCD enzyme positively correlated with IAA production (Figures 2A, D, and E).

IAA production is one of the most relevant mechanisms among bacteria associated with plants, especially in the roots (Botelho et al. 2019; 2023). The search for multifunctional plant growth-promoting microorganisms, especially bacteria, has intensified (Silva et al. 2022; 2023). Bacteria promoting plant

growth by more than one mechanism have already been used for bio-input production, such as *Azospirillum brasilense*. This species is best known for fixing N and producing IAA (Fukami et al. 2017). Thus, it can be used in association with other bacterial species, such as *Bradyrhizobium japonicum* (Torres et al. 2022).

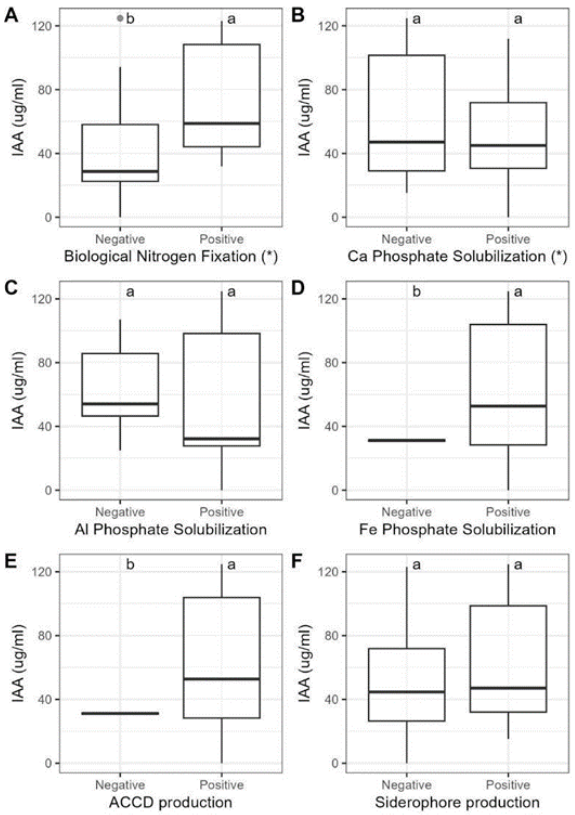


**Figure 2.** Comparison among IAA production and the other growth mechanisms tested in *Pseudomonas* spp. isolates.

## 5. Conclusions

The plant growth-promoting mechanisms (Fe and Al phosphates solubilization, siderophore, and ACCD enzyme productions), tested *in vitro,* were found in more than 50% of fluorescent *Pseudomonas* spp. isolates, indicating a significant community with these mechanisms regarding plant growth stimulation.

The isolates selected for field analysis (CBSAL02, CBSAL05, and CBSAL06) could solubilize Fe and Al phosphates and produce the ACCD enzyme and siderophores, except for the CBSAL05 isolate. In the field performance, the three isolates and *A. brasilense* increased the N content in the leaves compared to the control, suggesting stimulus for N acquisition by the plants.

The CBSAL06 isolate stimulated stalk diameter, number of grains per row, and productivity, standing out in this parameter, thus suggesting its potential as an inoculant.

**Authors' Contributions:** BOTELHO, G.R.: conception and design, acquisition of data, drafting the article and critical review of important intellectual content and final approval of the version to be published; BELEN, G.D.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article and critical review of important intellectual content and final approval of the version to be published; CYSNEIROS, V.C.: analysis and interpretation of data, drafting the article and critical review of important intellectual content and final approval of the version to be published; GUIMARÃES, A. G.: analysis and interpretation of data, drafting the article and critical review of important intellectual content and final approval of the version to be published.

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**Ethics Approval:** Not applicable

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