Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2023v44n4p1507

Nodulation and biological nitrogen fixation of common bean rhizobia from Santa Catarina Plateau soils

Nodulação e fixação biológica de nitrogênio de rizóbios de feijão de solos do planalto Catarinense

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Highlights

Seleção de novos rizóbios adaptados às condições edafoclimáticas da região.
Uso de mecanismos de promoção de crescimento para seleção dos rizóbios.
Eficiência dos rizóbios selecionados em diferentes genótipos de feijão.

Abstract

Curitibanos town is the third regular bean producer (*Phaseolus vulgaris* L.) in Santa Catarina. However, the regional production decreased due to problems such as the nitrogen fertilizers cost. Farmers do not use seed inoculation because of inoculant's rhizobia low performance and adaptation to edaphoclimatic conditions. Thus, 36 rhizobia were isolated, characterized, and evaluated by *in vitro* indole acetic acid (IAA) production and phosphate solubilization, as auxiliary tools to the rhizobia selection. Ninety-two percent of the isolates produced IAA and 64% solubilized calcium phosphate. The isolates selected for field trials during two consecutive years were RBZ14 and RBZ15. RBZ14 produced an IAA higher amount than strain CIAT899 (recommended for inoculants) and RBZ15 formed a lower amount. For phosphate solubilization, all showed similar performance. In the first year, the isolates increased leaf N contents in the TAA Dama cultivar. RBZ15 increased the productivity. In the second year, in the IPR Tuiuiú cultivar, the isolates also increased leaf N contents, and there was no difference at productivity but increments of 120 and 205 kg.ha^{-1} with RBZ14 and RBZ15 inoculations, respectively. Therefore, it suggested their potential for BNF in different genotypes and that evaluated mechanisms may help to the selection of a more efficient rhizobia.

Key words: Fabaceae. Bacteria. Efficiency. Adaptation. Mechanisms.

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Received: Mar. 04, 2023 Approved: Oct. 05, 2023

Resumo

Curitibanos é o terceiro produtor de feijão (*Phaseolus vulgaris* L.) do estado de Santa Catarina, Brasil. Porém, a produção tem sido reduzida por problemas como o custo dos fertilizantes nitrogenados. Os agricultores não utilizam a inoculação de sementes devido ao baixo desempenho dos rizóbios existentes em inoculantes, por dificuldade de adaptação dos microrganismos às condições edafoclimáticas da região. Por este motivo, 36 rizóbios foram isolados, caracterizados e avaliados pela produção *in vitro* de ácido indol acético (AIA) e solubilização de fosfato, como ferramentas adicionais para seleção de rizóbios mais eficientes. Noventa e dois por cento dos isolados produziram AIA e 64% solubilizaram fosfato de cálcio. Os isolados selecionados para testes de campo foram RBZ14 e RBZ15. RBZ14 produziu maior quantidade de AIA que CIAT899 (estirpe usada em inoculantes) e RBZ15 em menor quantidade. Para solubilização de fosfato os isolados RBZ14 e RBZ15 apresentaram desempenho semelhante. No primeiro ano, os isolados RBZ14 e RBZ15 aumentaram os teores de N foliar na cultivar TAA Dama. RBZ15 aumentou a produtividade. No segundo ano, na cultivar IPR Tuiuiú, os isolados RBZ14 e RBZ15 aumentaram os teores de N e não houve diferença na produtividade, mas incrementos de 120 e 205 kg.ha^{-1} com as inoculações de RBZ14 e RBZ15, respectivamente, sugerindo seus potenciais para FBN em diferentes genótipos. Observou-se também que a produção de AIA e a solução de fosfato podem auxiliar na seleção de rizóbios mais eficientes.

Palavras-chave: Fabaceae. Bactérias. Eficiência. Adaptação. Mecanismos.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most widespread legumes in the world, because it is a high protein and iron crop (Soares et al., 2006). Brazil, one of the major consumers (14.94 kg/inhab/year) is the third largest producer in the world, accounting for 13% of bean production (Food and Agriculture Organization of the United Nations [FAOSTAT, 2020]). At the 2021/2022 harvest, the Brazilian bean production was 3.0 million t. from a 2.92 thousand ha area (Companhia Nacional de Abastecimento [CONAB], 2023). Santa Catarina is the 5th state in the national bean production ranking, with 106 thousand t. (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina /Centro de Socioeconomia e Planejamento Agrícola [EPAGRI/CEPA], 2022). A big part of this comes from family farming, responsible for 65%. Curitibanos region, in Santa Catarina Plateau, had the highest production in the state. Nowadays, it is the fifth producer, with around 6,000 t at the 2021/2022 harvest (EPAGRI/CEPA, 2022). Although the Santa Catarina Plateau is still among the main bean producing regions, yield, and productivity have been limited by factors such as low prices to farmers, disease incidence, inputs prices, and especially fertilizers (EPAGRI/CEPA, 2022).

Nitrogen (N) is the main element for plant growth and development, but its soil availability is lower than demand, requiring N fertilization that increases production costs. In addition, inadequate management can result in environmental effects, mainly due to leaching processes. Economic and environmental costs of N fertilization can decrease using the symbiotic N-fixing bacteria. Rhizobia may ensure the N supply to the plant, enabling an important gain within ecosystems. Studies have shown that rhizobia inoculation significantly increased bean production (Brito et al., 2015). According to Oliveira and Sbardelotto (2011), the common bean productivity can reach from 2,500 kg ha^{-1} to 3,000 kg ha^{-1} with rhizobial inoculation. Soares et al. (2006) reported that rhizobia inoculation increased the bean productivity to 900 kg ha^{-1}. However, this association cannot supply all N demand to common bean (Brito et al., 2015).

Biological nitrogen fixation (BNF) efficiency depends on the host plant physiological features. Other factors can also limit it and its use. One is the genetic heterogeneity of bean cultivars (Milcheski et al., 2022). Furthermore, the survival and competitiveness of commercial inoculant bacteria compared to the soil rhizobia community, in addition to their adaptation to edaphoclimatic aspects, are also limiting factors. Currently, the commercial inoculant recommended for common beans in Brazil is produced with *Rhizobium tropici* adapted to tropical soils, resistant to high temperatures, soil acidity, and highly competitive. It may also form most of nodules, predominating over the rhizobia community in most soils (Martinez-Romero et at., 1991; Soares et al., 2006). Nevertheless, in the Curitibanos mesoregion, inoculation at common beans did not achieve satisfactory results due to the soil rhizobia competitive community or the low adaptability of inoculant populations to the regional edaphoclimatic characteristics. Usually, endogenous rhizobia communities are more competitive for root infection sites and, sometimes, less efficient in BNF.

Common bean promiscuity associated with the different taxonomic groups of rhizobia, can increase this effect (Gunnabo et al., 2021). However, this feature can be a strategy to select more efficient rhizobia (Brito et al., 2015; Wekesa et al., 2021), combined with other plant growth mechanisms that could improve BNF. Indole Acetic Acid (IAA) production and phosphate solubilization may stimulate nodulation and BNF processes (Rocha et al., 2018). Therefore, bacteria isolation from these soils can be an alternative to improve this interaction and, consequently, stimulate their use by farmers. Thus, this work's objective was to isolate, select, and evaluate the efficiency of endogenous common bean rhizobia from Curitibanos soils, which have other plant growth-promoting mechanisms that could stimulate BNF.

Materials and Methods

Isolation, authentication and characterization of bean rhizobia

Rhizobia isolation was carried out in a greenhouse experiment at the Curitibanos *campus* of UFSC (Universidade Federal de Santa Catarina), with soil from Dias Farm, at Horizolândia, Curitibanos county, in 2014. It was classified as Cambisol associated with Nitosol Bruno (Santos et al., 2018). Soil sampling was performed according to the (Comissão de Química e Fertilidade do Solo – RS/SC [2016]). Serial dilution was proceeded using 10 g of soil. Common bean seeds, cultivars IPR Tuiuiú, were previously disinfected by immersion in 96° GL alcohol for 1 min, followed by 3% sodium hypochlorite

(NaOCl) for 1.5 min. Finally, the seeds were washed ten times in sterile distilled water. Then, they were pre-germinated on absorbent paper moistened with sterile water, stored in clean plastic bags and incubated in the dark at 28 °C. After 48 h, seeds were transferred to sterile 500 mL glass pots. Each pot had an absorbent paper to support a seed and 300 mL of nutrient solution without N for plant growth (Norris & Date, 1976). Then, 1 mL of each soil dilution was inoculated per seed. Each treatment (dilution) had four replications. After the third trefoil emergence, ten nodules were removed from each plant. They were disinfested. After disinfestation, the nodules were carefully macerated in Petri dishes with a glass rod inside laminar flow. Suspensions were streaked on yeast mannitol agar (YMA) with Congo red (0.25% w/v) plates with pH 6.8 (Hungria, 1994) and incubated at 28 °C until colonies grew. The colonies were sub cultured on YMA Congo red plates until isolation and purification.

After purification, they were grown in five mL of liquid LB (Lurian Bertani) medium for 24 h at 28 °C. Then, 1 mL of each bacterial suspension was mixed with 1 mL of 80% glycerol solution and stored at -20ºC, with three replications for each isolated bacterium. Later, the isolates were grown in five mL of liquid LB for 24 h at 28ºC and inoculated onto seeds of bean cultivar IPR Tuiuiú previously disinfested, as described above. They were sown in tubes containing sterilized vermiculite and sand (2:1) mixture. Seedlings were irrigated daily with sterilized distilled water. After the third trefoil emergence, the nodules were collected, washed, counted, and stored in glass vials containing silica at room temperature. It was possible to perform Gram stain, acid or base production and growth time of each isolate for the phenotypic analyses. Last two tests were carried out in liquid YM containing bromothymol blue (0.5% w/v), based on Hungria (1994) and incubated at 28 °C. Medium pH changes were evaluated considering isolate growth time. Isolates that acidified medium changed their color to yellow.

The alkalized ones showed a bluish color. The isolates that did not change pH, kept medium green. For growth time, isolates that grew up 72 h after inoculation were considered fast growth. Those that grew four to five days were intermediate growth, and isolates that grew longer than six days were slow growth.

Two mechanisms of plant growth induction were tested: Phosphate solubilization and Indole Acetic Acid (IAA) production. Chagas et al. (2010) method determined Calcium phosphate solubilization. The isolates were grown in five mL YM liquid with bromothymol blue (pH 6.8) and incubated for 72 h. After growth, ten µL of each isolate suspension was transferred to plates containing the solubilization medium (Chagas et al., 2010). Each plate had four isolates inoculated at four equidistant points. After incubation, solubilization halo and colony diameters (Ø) were measured. The solubilization index (SI) formula proposed was SI=Ø halo/Ø colony. The following parameters were considered to SI: low solubilization: < 2; average: >2 and < 4; high solubility: > 4 (Chagas et al., 2010). The experimental design was Completely Randomized (CRD), with four replicates. The F statistic in the Analysis of Variance (ANOVA) and Scott-Knott average test were performed, at a significance 5%, using the RStudio system (R Core Team [R], 2023).

For the qualitative and quantitative analyses of IAA production, isolates were grown in five mL liquid LB (Luria Bertani) for 48 h at 28ºC. Later, ten µL of the pre-inoculum were transferred to new tubes containing five mL LB added 100 µL.mL^{-1} of tryptophan (0.005 g.mL^{-1}). Tubes were kept at 28 °C for 24 and 48 h. For qualitative analysis, 1 mL of bacterial suspension and 1 mL of Salkowski reagent (0.5 M solution of $FeCl_3$ in 7.9 M H_2SO_4). The mixture was left in the dark for 25 min. The pink color in the tubes indicated IAA production (Marchioro, 2005) that was quantified. Then, a standard curve was prepared by absorbances at 540 ηm (spectrophotometer - Bel sp 2000uv) of 100, 200, 300, 400 and 500 µg. mL^{-1} of IAA (Vetec) concentrations. Each concentration point had three replicates. Excel software verified the Standard curve R^2 to check its linearity. The experimental design was Completely Randomized (CRD), with three replicates. The F statistic in the Analysis of Variance (ANOVA) and Scott-Knott average test, at 5% of significance was performed, using the DEX/UFLA Sisvar 5.7 software (Ferreira, 2008).

Isolates inocula and field trials description

Two isolates (RBZ14 and RBZ15) were chosen for field trials based on their IAA production and phosphate solubilization capacity, as an additional mechanism to BNF. They were compared to *R. tropici* strain CIAT899 (SEMIA 4077), recommended for inoculant and used as standard. RBZ14 was selected because it produced IAA higher than CIAT899 and RBZ15 a lower amount. For phosphate solubilization, all showed similar performance.

The two field trials were conducted in the Experimental area of the Federal University of Santa Catarina (UFSC), Curitibanos campus, during 2018/2019 and 2019/2020. It is located at the coordinates 27°16'26.55" S and 50°30'14.41W, at approximately 1000 m altitude. According to the Köppen classification, region's climate is temperate, humid mesothermal and mild summers. During the growing season (November/March), the regional average temperature was 24 °C, and the average rainfall was 134 mm. The experimental area soil class is the Haplic Cambisol (Santos et al., 2018).

The isolates inocula was performed in two stages. First, they were grown in tubes containing 10 mL of liquid LB at 28 °C for 48 h. Second, 1 mL of bacterial suspensions was transferred to flasks containing 1 g of sterilized peat. These were kept for 72 h at 28 °C until sowing. For the control and N fertilization treatments, flasks with 1 g of peat received 1 mL of non-inoculated medium kept on the same conditions. The treatments were control (without inoculation or N fertilization); N fertilization (150 to 120 kg of N/ha); RBZ14 isolate; isolate RBZ15 and CIAT899 (SEMIA 4077 - commercial inoculant). Each treatment required 500 g of seeds that were placed into plastic bags, added inoculated or non-inoculated peat and 5 mL of sugar solution (10%). Isolate inoculum production was based on the proportion of seed, peat inoculant, and sugar solution recommended by manufacturers (50 kg, 150 g, and 300 mL, respectively). CIAT899 inoculation was carried out according to the manufacturer's recommendation.

Both experiments (2018/2019 and 2019/2020) evaluated BNF capacity, by the N contents in leaves and in grains and productivity. In the first trial, the experimental design was at Randomized Blocks (RBD), with five treatments and five replicates. The plot area had six m² (3.0 x 2.0 m), with four sowing lines 0.4 m apart and 0.10 m between seeds. Urea was the N fertilizer and distributed at two times: 19 DAS (Days After Sowing) and stage V4, 35 DAS. Nutrients P and K (triple superphosphate and potassium chloride) were supplied 19 DAS, according to soil analysis (Table 1) and the recommendations of the Fertilization and Liming Manual for the states of Rio Grande do Sul and Santa Catarina, for the productivity of 1,500 kg.ha^{-1} to both experiments. For the first trial, the cultivar was TAA DAMA, brown grain, average life cycle of 89 days. For the second experiment, the cultivar was IPR Tuiuiú, black grain, average life cycle of 88 days.

Table 1
Experimental area soil analysis

	First trial (2018/2019)	Second trial (2019/2020)
pH $CaCl_2$	4.30	4.80
P (mg.dm^{-3})	40.12	6.63
K (mg.dm^{-3})	89.70	62.56
Ca (cmolc.dm^{-3})	3.43	2.30
Mg (cmolc.dm^{-3})	2.18	1.83
Al (cmolc.dm^{-3})	1.47	2.11
H + Al (cmolc.dm^{-3})	12.13	12.13
MO g/dm^3	61.28	49.80
SB (cmolc.dm^{-3})	5.84	4.29
V (%)	32.50	26.13
CTC pH7 (cmolc.dm^{-3})	17.97	16.42
Fe (mg/dm^3)	100.2	53.20
Mn (mg/dm^3)	39.62	13.50
Cu (mg/dm^3)	9.50	7.80
Zn (mg/dm^3)	1.70	1.20

Analysis provided by the Agricultural Department UFSC Curitibanos. Sampling from 20 cm deep.

The leaves were gathered during the crop's full flowering to quantify N leaf content, which occurred 48 DAS. Each sample consisted of posterior flower leaves of ten plants in each plot. They were dried at 65 °C until constant weight. The N analysis was determined by the Kjeldahl method (Tedesco et al., 1995). Productivity and N quantification in grains were evaluated at 98 DAS in the physiological maturity. Thirty plants from the inside plot area (2.0 x 1.5 m) were collected, and grain moisture was measured by the Motomco model 999ESI moisture meter. Grain weight was adjusted to 13% moisture, and productivity was estimated (kg.ha^{-1}). N grain contents were determined by the Kjeldahl method (Tedesco et al., 1995). The F statistic in the Analysis of Variance (ANOVA) and Scott-Knott means method compared data at a 5% significance level, by the SISVAR version 5.7 (Ferreira, 2008). The second experiment was developed in Randomized Block Design (RBD), in the same experimental area as the first trial, with six treatments and four replicates. Treatments were RBZ14 isolate; RBZ15 isolate; strain CIAT899 (SEMIA 4077 commercial inoculant); full N fertilization – 120 kg.ha^{-1} of N (100%); half N fertilization – 60 kg.ha^{-1} of N (50%), and control without inoculation or nitrogen fertilization. N leaves, grain contents, and productivity determination are described above. The F statistic in the Analysis of Variance (ANOVA) and Tukey's test mean compared data, at 5% of significance using the SISVAR software version 5.7 (Ferreira, 2008).

Results and Discussion

In vitro isolates characterization

Table 2 shows phenotypic assessments and *in vitro* promotion mechanisms of 36 isolates, after isolation and authentication procedures. All isolates were Gram-negative, fast-growing and 67% of them acidified the medium, like the standard strain, CIAT899. Most rhizobia group species are Gram-negative and when striated in YMA medium with Congo Red and incubated in the dark, they do not absorb the dye, presenting whitish and gummy colonies (Hungria, 1994). In general, fast-growing rhizobia tends to acidify the YMA medium plus bromothymol blue, turning it yellow *in vitro* isolates characterization (Martins et al., 1997), similar to our results (Table 2). According to Tan and Broughton (1982), the preferential use of sugars by fast-growing rhizobia promotes the excretion of organic acids, causing a reduction in pH. All the characteristics described were observed in the isolates, including mucus production, quite common among colonies of rhizobia (Martins et al., 1997). Fast growth capacity can imply more survival ability, adaptability, and rhizosphere competitiveness (Martins et al., 1997), increasing the stable rhizobia to these environments.

Thirty-three isolates (92%) produced IAA (Table 2). Five distinct groups were observed based on statistical analysis. Seventeen bacteria (47%) originated more IAA than the standard strain, CIAT899 (>75 µg.mL^{-1}). These 17 were split into three groups. The first had isolates that produced more than 136.46 µg.mL^{-1}. The second group produced between 111. 18 and 116. 39 µg.mL^{-1}, and the third one between 94.70 and 102. 36 µg.mL^{-1}. Six bacteria produced IAA like the CIAT899 (between 63.93 and 82.16 µg.mL^{-1}). Eleven isolates had between 31.81 and 57.50 µg.mL^{-1}. IAA was not detected in the RBZ16, RBZ17, RBZ18 and RBZ34 isolates. Auxin production is the most described mechanism to explain the effect of rhizobacteria on plant growth (Jaiswal et al., 2021). It can be produced from tryptophan, present in root exudates. One of the functions of auxins in plants is root growth (Taiz & Zeiger, 2004). The results showed that most of isolates produced IAA (Table 2), and there was a wide variation in its production (from 31.87 to 153.82 µg.mL^{-1}), which matched with other reports (Raklami et al., 2019). Raklami et al. (2019) when analyzing strains of *Acinetobacter* sp., *Rahnella aquatilis* and two *Ensifer meliloti* rhizobia obtained values between 38.08 and 290.64 µg mL^{-1}, and rhizobia were the biggest producers (112.43 to 290.64 µg.mL^{-1}). These values were like most of the isolates' production (Table 2).

Table 2
Endogenous common bean rhizobia isolate characterization

Isolate	Growing time	Gram test	pH*	IAA production (µg mL^{-1})	Phosphate solubilization (SI) **
CIAT899	Fast	-	Acid	74.04d	1.77a
RBZ01	Fast	-	Alkaline	70.76d	1.77a
RBZ02	Fast	-	Alkaline	99.86c	1.63b
RBZ03	Fast	-	Alkaline	64.58 d	1.50b
RBZ04	Fast	-	Acid	46.39e	0.00d
RBZ05	Fast	-	Acid	97.43c	0.00d
RBZ06	Fast	-	Alkaline	99.37c	1.33b
RBZ07	Fast	-	Acid	73.54d	1.80a
RBZ08	Fast	-	Acid	102.36c	2.10a
RBZ09	Fast	-	Alkaline	82.16d	0.00d
RBZ10	Fast	-	Acid	152.08a	1.80a
RBZ11	Fast	-	Acid	136.67a	1.73a
RBZ12	Fast	-	Acid	75.90d	1.77a
RBZ13	Fast	-	Acid	149.17a	1.47b
RBZ14	Fast	-	Acid	153.26a	1.73a
RBZ15	Fast	-	Acid	57.50e	2.20a
RBZ16	Fast	-	Alkaline	nd	0.00d
RBZ17	Fast	-	Alkaline	nd	0.00d
RBZ18	Fast	-	Acid	nd	0.92c
RBZ19	Fast	-	Alkaline	42.71e	0.00d
RBZ20	Fast	-	Acid	44.76e	0.00d
RBZ21	Fast	-	Acid	153.82a	1.50b
RBZ22	Fast	-	Acid	63.96d	0.00d
RBZ23	Fast	-	Acid	43.33e	0.00d
RBZ24	Fast	-	Acid	99.51c	1.63b
RBZ25	Fast	-	Alkaline	49.72e	1.60b
RBZ26	Fast	-	Acid	47.22e	0.00d
RBZ27	Fast	-	Alkaline	47.22e	0.00d
RBZ28	Fast	-	Alkaline	57.01e	1.70a
RBZ29	Fast	-	Acid	148.89a	1.60b
RBZ30	Fast	-	Acid	94.79c	1.57b
RBZ31	Fast	-	Acid	115.35b	1.90b
RBZ32	Fast	-	Acid	116.39b	1.53b
RBZ33	Fast	-	Acid	136.46a	1.47b
RBZ34	Fast	-	Acid	nd	0.00d
RBZ35	Fast	-	Alkaline	111.18b	0.00d
RBZ36	Fast	-	Alkaline	31.88e	0.00d

Fast – Growth up to 72 h; (-) Negative; * pH modification of bromothymol blue YMA medium; ** SI – solubilization index calculated after three days of inoculation. CV (Coefficient of variation) = 13.10%; Means followed by the same letters do not differ by the Scott-Knott method at a 5% significance level. nd – not determined.

Twenty-three isolates could solubilize calcium phosphate, representing 64% of the total (Table 2). There was no significant difference at solubilization halos to each isolate along the evaluations (3, 6 and 9 days after inoculation). On the third day, the halos were clearer and were chosen for statistical analysis. Halos' sharpness allowed more efficient measurement, either by ruler measurements or by software. There were four statistical groups (Table 2). The first isolates had a higher SI, like CIAT899 (between 1.70 and 2.20). In this group, bacteria were selected for field trials (RBZ14 and RBZ15). A group was formed only by the isolate RBZ18 (SI= 0.92). The third one contained those with SI between 1.33 and 1.63, and finally, isolates that did not have solubilization halo. Regarding P-solubilization, it was observed that highest SI group represented 43% of the isolates (Table 2), suggesting an expressive population among rhizobia in the region. This mechanism has been reported among rhizobia communities (Chagas et al., 2010; Jaiswal et al., 2021). Also, it can help bacterial selection to inoculants, when enhancing BNF by increasing efficiency and stimulating their use, especially for bean cultivation. On third day of incubation, in vitro analysis indicated that solubilization halos were clearer, and sharpness allowed for more efficient measurement. During the first three days of inoculation, Chagas et al. (2010) observed clearer solubilization, and they classified them based on SI while evaluating rhizobia isolates for phosphate solubilization from Amazonian soils. They observed that most of the isolates had SI below 2.0, which corroborated to our data (Table 2). Organic acid production is considered the principal mechanism of solubilization by soil microorganisms (Chagas et al., 2010). Among ten isolates showing the highest SI, eight (80%) acidified the YMA medium (Table 2). In soils with low P availability, the rhizobia can solubilize P in the root region by acidification and gluconic acid synthesis (Jaiswal et al., 2021). Solubilization ability by rhizobia (or rhizobacteria) can be used as an additional mechanism for their selection since P fertilization and its losses are recent economic and environmental challenge, too.

A rhizobia community capable of producing IAA, and solubilize P and consequently stimulating BNF, encouraged preliminary studies with some isolates in the field. RBZ14 and RBZ15 were selected. It was possible to observe that RBZ15 produced approximately three times less IAA than RBZ14, and both were high P solubilizers (Table 2).

Field trials isolate performance

Two experiments were conducted during consecutive seasons (2018/2019 and 2019/2020) with different grain cultivars (brown bean and black bean), because both are sowed in the region. The first trial was conducted during the 2018/2019 harvest, using the brown (pinto) bean cultivar TAA DAMA. The F statistics of variance analysis indicated a significant response between treatments (control, RBZ14, RBZ15, and CIAT899, and N fertilization) for leaf nitrogen contents and productivity (Table 3). RBZ15 inoculation had N contents statistically equivalent to the N fertilizer treatment, suggesting its ability to fix N efficiently, which was translocated to the shoot. Control treatment, RBZ14 and CIAT899 did not differ from each other and had the lowest averages (9.40 to 17.90 g.kg^{-1}).

Nevertheless, RBZ14 and CIAT899 inoculations increased N contents at 90% and 30%, respectively, compared to the control. The leaf N contents at CIAT899 inoculation suggested some symbiotic difficulties for the strain, probably due to the regional edaphoclimatic conditions. Night temperatures in the Curitibanos region can be significantly lower than daytime, ranging from 16 °C to 25 °C, respectively (Climatempo, 2021), when compared to the CIAT889 original isolation site (Martinez-Romero et al., 1991). The average annual temperature was higher throughout the year, with no marked variations during the day. This factor may be one of the barriers to bacterial adaptation, nodulation and BNF efficiency. Trials by Hungria et al. (2000) showed CIAT899 could match, in terms of leaf nitrogen, to nitrogen fertilization, indicating its efficiency in warmer regions. Other possibilities were higher competition for infection sites by endogenous soil rhizobia, lack of synchrony between strain N supply and plant demand due to fast nodule senescence, or BNF reduction after flowering that needs to be considered and further analyzed.

Table 3
Endogenous rhizobia inoculation performance in common bean cultivars sown in Curitibanos (SC) region

Treatments	Leaf nitrogen ss ($g.kg^{-1}$)		Grain nitrogen contents ($g.kg^{-1}$)		Productivity ($kg.ha^{-1}$)	
Cultivars	**TAA Dama**	**IPR Tuiuiú**	**TAA Dama**	**IPR Tuiuiú**	**TAA Dama**	**IPR Tuiuiú**
Control	9.40b	11.09C	14.00a	15.80A	1.563d	1.239B
CIAT899	12.20ab	11.92C	16.80a	16.90A	1.796c	1.278AB
RBZ14	17.90ab	16.09B	17.20a	16.90A	1.756cd	1.413AB
RBZ15	22.30a	17.16B	16.20a	17.50A	2.348b	1.484AB
N fertilization	20.08a	23.81A	19.80a	18.60A	2.627a	1.741A
CV%	17.16*	8.12	20.14	9.91	7.78	14.14

TAA DAMA – 2018/2019 harvest; IPR Tuiuiú – 2019/2020 harvest. Means followed by the same letter do not differ using the Scott-Knott test at 5% significance: Lower case letter for TAA Dama and capital letter for IPR Tuiuiú. *Means were transformed.

Regarding grain N contents, there was no significant difference (Table 3). Similar results were observed by Fidelis et al. (2019), who did not observe statistically significant differences among bean grains. The productivity showed differences among treatments, ranging from 1.563 to 2.627 $kg.ha^{-1}$ (Table 3). Control obtained the lowest productivity, but still like the Brazilian average, according to CONAB (2023), which described 1.513 $kg.ha^{-1}$. It might occur due to the high OM (Organic Matter) contents (61.28 $g.dm^{-3}$), according to soil analysis (Table 1), providing part of N supply or even, due to endogenous rhizobia since *P. vulgaris* is non-specific to rhizobia. RBZ14 inoculation productivity had no difference from the control but increased by 12.34%. It was also statistically equivalent to CIAT899 inoculation, differencing only 2.22% (40 kg). This result suggested that RBZ14 was as efficient as CIAT899 to BNF in Curitibanos (SC) conditions in spite of adaptation. Soares et al. (2006) had similar results, evaluating endogenous rhizobia from Perdões (MG), in which UFLA 02-100, UFLA 02-86 and UFLA 02-127 isolates achieved productivity like CIAT899.

In the RBZ15 inoculation, there was a significant productivity increase, compared to CIAT899 (30.73%). The isolate reached the highest average among inoculated treatments (Table 3). Hungria et al. (2000) observed similar results in a study with isolates (PRF 35, PRF 54 and PRF 81) endogenous to Paraná (Brazilian state) soils. RBZ15 inoculation productivity was lower than nitrogen fertilization (Table 3), which was expected since bean cultivars were selected for high nitrogen availability.

The second trial was in 2019/2020 harvest, using the black bean cultivar IPR Tuiuiú. The F statistic of the analysis of variance showed that there was difference among treatments for leaf nitrogen contents and productivity (Table 3). CIAT899 inoculation and control had the lowest N amounts, confirming the strain adaptation difficulty to the regional soil and climate traits, observed at the first experiment.

Leaf N contents in RBZ14 and RBZ15 inoculations were higher than CIAT899, but RBZ15 was not equivalent to N fertilization like in first trial. Again, there was no difference among treatments for grain N contents like in the first essay. There were no significant variations in the grain N contents to the cultivars tested, suggesting low sensitivity as a parameter.

The control showed the lowest average with 1.239,40 kg.ha^{-1} for the productivity, despite there was no difference from inoculated treatments (Table 3). However, there was an increase of 174 kg.ha^{-1} with RBZ14 (14%) inoculation, 245 kg.ha^{-1} (19%) with RBZ15 inoculation, and only 39 kg.ha^{-1} (3%) with CIAT899 inoculation. Isolates' adaptation to edaphoclimatic regional features may improve their performances. Soares et al. (2006) compared bean cultivar Talismã inoculated with rhizobia isolates, strain CIAT899 and N fertilization in Minas Gerais state. They also observed that the productivity at isolates inoculation was statistically equal to CIAT899 and N fertilization.

The great diversity found among endogenous rhizobia in different regions (Efstathiadou et al., 2021; Gunnabo et al., 2021), associated with plant growth stimulating mechanisms, such as IAA synthesis and P-solubilization (Sottero et al., 2006), may allow selection of more efficient BN-fixers and adapted bacteria. It is important to emphasize that most of the nodulating bean rhizobia belongs to alpha-Proteobacteria, especially to genera *Rhizobium* and *Pararhizobium* (Shamseldin & Velázquez, 2020). Other genera, including non-nodulating bacteria, have also been described as nodules colonizers (Martínez-Hidalgo & Hirsch, 2017). A large part of this bacteriome can produce IAA, solubilize P, among other mechanisms, emphasizing the importance of comprehending this community dynamics, to increase strategies to isolate efficient new rhizobia.

RBZ14 and RBZ15 produced higher and lower IAA amount than CIAT899, respectively (Table 2). RBZ15 stood out in productivity among the inoculated bacteria in the first trial. It may suggest that lower IAA production can be more appropriate as an additional criterion for bacteria selection, at least to brown (pinto) beans for these edaphoclimatic traits. Leaf N contents were also highest with RBZ15 inoculation. For this cultivar, there was no significant difference between inoculated and N-fertilized treatments (Table 3). In this case, it may suggest that the lowest IAA production was more relevant for greater RBZ15 effectiveness since the isolates and strain had similar performances for P solubilization (Table 2).

For the IPR Tuiuiú cultivar (second trial), the potential relevance of IAA production was not so evident. Leaf N contents with RBZ14 and RBZ15 inoculations were higher, but there was no difference between them (Table 3). Regarding productivity, there was no difference among inoculated treatments, but there was an average increase of 170.5 kg.ha^{-1} with isolates inoculations.

The results suggested that growth-promoting mechanisms can stimulate BNF and some of them, more relevant to the symbiotic effectiveness would be associated to the common bean genotype (Milcheski et al., 2022). Therefore, they require further studies.

Conclusions

All isolates were Gram-negative and fast-growing. Ninety-two percent of endogenous rhizobia produced IAA and 64% solubilized Ca phosphate similar to or higher than the CIAT899 strain suggesting, a significant community in the regional soil.

In the field trials, the CIAT899 strain had low performances, suggesting adaptation barriers. RBZ15 isolate, a lower IAA producer, was as efficient as the N fertilization for leaf N contents and stood out in productivity among inoculated treatments, for brown (pinto) bean cultivar, suggesting the mechanism might influence BNF.

For black bean cultivar, RBZ15 and RBZ14 inoculations showed higher leaf N contents than CIAT899. For productivity, the isolates and strain inoculation effects were equivalent. However, RBZ14 and RBZ15 isolates provided an average increase of 16.5%. No prevailing effect of one mechanism on BNF was observed, but both seemed to stimulate it.

The results suggested that the plant growth-promoting mechanisms may have positively influenced the RBZ14 and RBZ15 performances in field trials, indicating relevance to the symbiotic process and interaction with different common bean genotypes.

Acknowledgments

Ww thank the Universidade Federal de Santa Catarina, farmers of Curitibanos town, especially Mr. Dias and Embrapa – Arroz e feijão, especially researcher Dr. Enderson Ferreira.

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