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CROP PRODUCTION

Yield increase of corn inoculated with *Pseudomonas thivervalensis* strain SC5 in Brazil

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ABSTRACT: Previous research has demonstrated the ability of isolate *Pseudomonas thivervalensis* SC5 to express the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACC), which regulates ethylene levels, one of the most important phytohormones in the regulation of plant growth and development. Thus, the present study evaluated the agronomic efficiency of a biological conditioner based on *P. thivervalensis* SC5 in the growth and productivity increases of corn in Brazil. It was found that corn was highly responsive to the inoculation of *P. thivervalensis* SC5, with increments ranging from 10.1 to 40.6% in the production of dry shoot biomass (DSB) compared to the control, while for grain yield the increments ranged from 9.0 to 27.8%. The increments are related to the levels and accumulations of N and P in the shoots of the plants. This suggested the participation of *P. thivervalensis* SC5 in mechanisms of soil modulation and nutrient acquisition. The inoculation of *P. thivervalensis* SC5 provided average increments in FDA hydrolysis ranging from 16.7 to 47.4% compared to the control, confirming the ability of this strain to increase the supply of nutrients to plants. Therefore, it is concluded that *Pseudomonas thivervalensis* SC5 participates in key mechanisms in the soil-plant system, with a consequent improvement in soil quality and other plant-related parameters.
Key words: nitrogen fertilization, soil conditioning, corn yield.

Aumento da produtividade do milho inoculado com *Pseudomonas thivervalensis* cepa SC5 no Brasil

RESUMO: Os microrganismos promotores do crescimento de plantas (MPCP) proporcionam uma série de efeitos diretos e indiretos sobre o crescimento das plantas, sendo muitos dos mecanismos associados com melhorias das condições físicas, químicas e biológicas do solo, podendo ser classificados como condicionadores de solo. Desta forma, o objetivo do presente estudo foi avaliar a eficiência agronômica de um condicionador biológico a base de *Pseudomonas thivervalensis* SC5 no desenvolvimento e produtividade da cultura do milho no território brasileiro. Ao final do ciclo da cultura foi determinada a produção de biomassa da parte aérea, o teor e acúmulo de N e P na parte aérea das plantas, o rendimento de grãos e a atividade enzimática do solo por meio da quantificação da hidrólise do Diacetato de Fluoresceína (DAF). A cultura do milho foi altamente responsiva à inoculação com o produto a base de *P. thivervalensis* SC5, com incrementos variando de 10,1 a 40,6% na produção de biomassa seca da parte aérea (MSPA) e de 9,0 a 27,8% para rendimento de grãos comparativamente ao controle. Esses incrementos proporcionados pelo condicionador aqui testado foram relacionados com os teores e acúmulos de N e P na parte aérea das plantas. Além disso, a inoculação de *P. thivervalensis* SC5 proporcionou aumentos de 16,7 a 47,4% na atividade enzimática global do solo (DAF), confirmando a capacidade da referida cepa em atuar no aumento da atividade biológica do solo que reflete no aumento do fornecimento de nutrientes para a cultura. Conclui-se que a cepa *Pseudomonas thivervalensis* SC5 apresenta característica condicionadora do solo por meio de distintos mecanismos de atuação no sistema solo-planta, com consequente melhoria da qualidade do solo e aumento no rendimento de culturas de interesse agrícola, incluindo o milho.
Palavras-chave: fertilização nitrogenada, condicionador de solo, rendimento de grãos de milho.

INTRODUCTION

The soil is a thriving environment for microorganisms of all kinds. Regardless of the number of microorganisms in a particular soil sample, they may affect plants in one of three ways: beneficial, harmful or neutral. The numbers of microorganisms in soil vary between different soil types and edaphoclimatic conditions, with bacteria generally being the most numerous. There can be more than 10^8 cells of bacteria and 10^5 cells of fungi in a gram of dry soil (WHITMAN et al., 1998). Growth of microbial populations and their action on soils are dependent on the interaction between plant species and soil (GRAYSTON et al., 1998; YADAV et al., 2023). Both the number and the type of microorganisms are influenced by the soil conditions including temperature, moisture, the presence of salts and other chemicals, as well as by the interaction between soil type, plant species and its rhizosphere localization (MARSCHNER et al., 2001; GLICK, 2012; TAHAT et al., 2020). Furthermore, the diversity and distribution of those organisms vary greatly within the soil matrix, being influenced primarily by the distance from the plant root system.

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Innumerous bacteria and fungi have been described as promoters of plant growth (PGPM). Among them are the classical examples of the nitrogen fixers (*Rhizobium, Bradyrhizobium, Azospirillum, Bacillus, Pseudomonas,* etc.), the phosphate solubilizers (arbuscular mycorrhizal fungi, *Bacillus*, *Pseudomonas, Streptomyces,* etc.), the siderophore producers (*Burkholderia, Pseudomonas*, etc.) and the biocontrol agents (*Trichoderma, Verticillium,* etc.) that directly or indirectly improve plant growth (DE SOUZA et al., 2015; VEJAN et al., 2016; OLANREWAJU et al., 2017; KUMAR et al., 2018; NASCIMENTO et al., 2021). Among various PGPM, *Bacillus* and *Pseudomonas* are the most abundant genera in the rhizosphere of any given ecosystem (PODILE & KISHORE, 2006; KUMAR et al., 2018).

The modes of action of PGPM to improve plant growth include (but are not restricted to): (1) the synthesis of specific compounds of interest to plants (DOBBELAERE et al., 2003; ZAHIR et al., 2004; DOBBELAERE & OKON, 2007); (2) a facilitation in the absorption of nutrients from the soil (GARCIA et al., 2004a, 2004b; ÇAKMAKÇI et al., 2006); and (3) a mitigation or reduction in the effects of pests and/or pathogens (JETIYANON & KLOEPPER, 2002; RAJ et al., 2003; GUO et al., 2004; SARAVANAKUMAR et al., 2008). The mechanisms by which PGPM stimulate plant development include (HAYAT et al., 2010): (1) production of vital enzymes, such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, capable of reducing the level of plant ethylene, thereby increasing root length, growth and plant development (GLICK & PENROSE, 1998; LI et al., 2000; BELIMOV et al., 2009; GAMALERO & GLICK, 2015); (2) ability to synthesize plant growth hormones such as auxins (indole acetic acid-IAA, abscisic acid-ABA), gibberellins (gibberellic acid) and cytokinins (DANGAR & BASU, 1987; PATTEN & GLICK, 2002; DOBBELAERE et al., 2003; DEY et al., 2004); (3) ability to fix nitrogen symbiotically (KENNEDY et al., 1997; 2004); (4) capacity to antagonize phytopathogenic bacteria by the production of siderophores, ß-1,3-glucanases, chitinases, antibiotics, fluorescent pigments and cyanide (PAL et al., 2001; GLICK & PASTERNAK, 2003; ZHANG et al., 2009); (5) capability to solubilize and mineralize nutrients, especially mineral phosphates (RICHARDSON, 2001; BANERJEE & YASMIN, 2002; HAYAT et al., 2010); (6) greater resistance to drought (ALVAREZ et al., 1996), salinity, flooding (SALEEM et al., 2007; ALI et al., 2014) and oxidative stress (STAJNER et al., 1995; 1997); and (7) the production of water-soluble B vitamins such as niacin, pantothenic acid, thiamine, riboflavin, and biotin (MARTINEZ-TOLEDO et al., 1996; SIERRA et al., 1999; REVILLAS et al., 2000).

Plant growth enhanced by PGPM is quantified as an increase in seedling emergence, vigor, biomass, proliferation of root system and yield in various plant species. However, the effects of PGPM in crop productivity varies under laboratory, greenhouse and field trials and the main reason for that is the enormous variability that soil and climate can have from one location to another, one season to another, one crop to another, among others. More likely, plant growth promoting traits do not work independently of each other but additively, in other words, multiple mechanisms, such as phosphate solubilization, dinitrogen fixation, ACC deaminase and antifungal activity, IAA and siderophore biosynthesis, etc., are responsible for the plant growth promotion and increased yield.

With that in mind, this research evaluated the field agronomic efficiency of an isolate of *Pseudomonas thivervalensis*, strain SC5 (ACC deaminase, phytohormones and siderophore producer, phosphate solubilizer, etc.), in combination to different nitrogen fertilization levels, in the growth and yield of corn in four different edaphoclimatic locations in Brazil.

MATERIALS AND METHODS

Experiments were carried out in the field to validate the efficiency and viability of the tested strain, following the protocols imposed by MAPA (Ministry of Agriculture, Livestock and Supply) for product registration of plant growth promoting microorganisms/soil conditioners, following the IN SDA 13 from 03/25/2011 (BRASIL, 2011), IN SDA 25, from 28/07/2009 (BRASIL, 2009), and IN SDA 53, from 10/24/2013 (BRASIL, 2013).

Among the main requirements for the registration of microbial inoculants/soil conditioners, there is the need to demonstrate the agronomic efficiency of the product in at least four locations of the country with distinct edaphoclimatic conditions. For this, four representative locations with distinct edaphoclimatic characteristics were selected (Tables 1 and 2). Experiments were conducted in the 2019/2020 crop cycle (October 2019 to June 2020), considering the planting dates, cultivars and agricultural driving practices of each tested location.

Experiments were implemented in a randomized block design with four treatments. Three treatments received 50% of the recommended

Table 1 - Information on planting, harvesting, cultivar and fertilization employed in each location.

State/ municipality	Planting date (2019/2020)	Harvesting date (2020)	Plant density (working area)*	Cultivar	---------------Fertilization---------------	
					Planting (N-P-K)	45 days after planting (N-P-K) §
SC - Mafra (26°13'28.8"S 49°42'30.0"W)	Oct 08 (2019)	Apr 08	168	30R50VYH	07-34-11 350 kg/ha	36-00-12 450 kg/ha
SP - Jundiaí (23°6'47.4"S 47°0'52.2"W)	Nov 19 (2019)	May 13	117	AG8061PRO2	08-20-10 413 kg/ha	26-00-07 376 kg/ha
SC – Canoinhas (26°11'35.5"S 50°37'04.0"W)	Jan 07 (2020)	May 21	178	Agroeste 1551 PRÓ 2	14-16-10 150 kg/ha	45-00-00 170 kg/ha
RS - Paraíso do Sul (29°43'38.48"S 53°7'6.45"W)	Dec 23 (2019)	Jun 16	79	MPA 01 Crioulo	No fertilization†	45-00-00 300 kg/ha

*Density varied according to cultivar. However, evaluations were based on the same number of plants per working area (30 plants).
§ For treatment 100% N (N100-NI).
† Planting after *Nicotiana tabacum*.

nitrogen for the crop: 1) non-inoculated (N50-NI); 2) inoculated with the commercial product NITRO 1000 (N50-NITRO 1000), which contains *Azospirillum brasilense* at a concentration of 2.0 x 10^8 viable cells per mL; and 3) inoculated with *Pseudomonas thivervalensis* SC5 (N50-SC5) at a concentration of 1.0 x 10^8 viable cells per mL. The fourth treatment received 100% of the recommended nitrogen and was not inoculated (N100-NI). The experiments were conducted with 10 replications each.

The experimental plots (40 plots per location) were assembled with an area of 50 m^2 (4 m x 12.5 m), occupying a total area of 2,214 m^2 per location (already considering 1 meter spacing between plots). The working area of each plot consisted of the central 20 m^2 portion of each plot (5 lines spaced by 0.5 m x 10 linear meters).

The tested product, under the trade name SC5 (registered under No. SC 002419-8.000001), is composed of 1.0 x 10^8 viable cells per mL (minimum) of the PGPM *Pseudomonas thivervalensis* strain SC5. The product was characterized and its purity certified. The number, the exclusive presence of cells of *P. thivervalensis* and the strain ID was also determined in this study in the laboratory of Microorganisms and Biotechnological Processes at UFSC. The identification approach considered characteristics of the CFU in PAF (*Pseudomonas* Fluorescence

Table 2 - Soil analysis of the experimental areas prior to the implementation of the experiments.

State/ municipality	Clay	-pH-	O.M.	--P--	--K--	-Ca-	-Mg-	--Al--	H+Al	CEC	-BS-	--V--
	%	H_2O	%	----mg/dm^3----		-------------------cmolc/dm^3-------------------					-	%
SC/Mafra	16	5.6	2.8	43.7	102.2	4.9	1.2	0.0	4.2	10.6	6.4	60.2
SP/Jundiaí	24	6.0	1.7	9.5	162.6	2.5	1.7	0.0	2.1	6.7	4.6	68.7
SC/Canoinhas	32	5.6	2.5	11.2	376.2	6.1	3.1	0.0	3.0	13.2	10.2	77.2
RS/Paraíso do Sul	9	5.5	1.4	156.1	118.8	2.4	1.0	0.0	2.8	6.5	3.7	57.0

O.M. – Organic Matter; P - Phosphorous; K - Potassium; Ca - Calcium; Mg - Magnesium; Al - Aluminum; H + Al – Potential Acidity; CEC – Cation Exchange Capacity (pH 7.0); BS – Bases Sum; V – Bases Saturation.

Agar) medium (KINGS et al., 1954). *Pseudomonas thivervalensis* has a very distinct appearance in PAF, with brilliant CFU that present fluorescence when exposed to a dark light. Quantification and purity were done using the decimal dilution series approach, and plating was done using PAF medium. All plates were incubated at 28 °C for at least 48 hours prior to any evaluation.

The seeds were inoculated by adding the inoculant directly to the seeds at the time of planting. The recommended amount of inoculant tested in the study was 100 mL/60,000 seeds. For the inoculated treatments, seeds were homogenized with the inoculant with the aid of plastic containers previously disinfected.

Crop management adopted in each location is described in table 3. For two of the evaluated states (RS and SC), seeds were treated with specific fungicides before planting. In addition to seed treatment, fungicides were also used during the crop cycle in the state of RS. The choice of products was based on the fungal disease identified in each evaluated location. Weed and insect control were carried out using specific herbicides and insecticides for each situation, respectively.

All fertilization procedures (based on soil analysis and expected corn yield) (SBCS, 2016) and crop treatment followed the recommendations for each location. The experiments were conducted until the plants completed the grain pre-maturation cycle: grains were in the kernel dough to kernel dent stages (R4-R5), that is, the phase immediately before physiological maturation (MAGALHÃES & DURÃES, 2006).

Table 3 - Edaphoclimatic characteristics, products for seed treatment and herbicides used in each location.

State/municipality	Soil type	Average temperature (°C) / According to Köppen	Accumulated rainfall (mm/cycle)[4]	Products used for seed treatment	Post-emergence herbicides
SC/Mafra	DHC[1]	19.5 / Cfb	668.2	Derosal Plus (Carbendazine + Thiram) Maxim XL (Fludioxonil + Metalaxyl−M) K-Obiol 25 EC (Deltametrine) Actellic 500 EC (Pyrimiphos-methyl)	Roundup (Glyphosate) Soberan (Tembotrione) Atrazina (Atrazine)
SP/Jundiaí	DRYU[2]	20.9 / Cfa	730.0	Maxim Advanced (Fludioxonil + Metalaxyl−M + Thiabendazole) K-Obiol 25 EC (Deltametrine) Actellic 500 EC (Pyrimiphos-methyl) Haiten (Polyoxyethylene alkyl phenol ether)	Roundup (Glyphosate) Posmil (Atrazine)
SC/Canoinhas	DRL[3]	18.3 / Cfb	236.5	Maxim XL (Fludioxonil + Metalaxyl−M) Poncho (Clotianidine) Cruiser 350 FS (Tiametoxan)	Roundup (Glyphosate)
RS/Paraíso do Sul	DRYU[2]	22.5 / Cfa	708.6	Maxim XL (Fludioxonil + Metalaxyl−M) Sumigran (Fenitrotione)	Not treated

[1]Dystrophic Haplic Cambisol; [2] Dystrophic Red Yellow Ultisol; [3] Distrophic Red Latosol.

[4]Precipitation data were collected at Epagri-Ciram meteorological stations for the municipalities of Mafra (26º13'28"S 49º48'43"W) and Canoinhas (26º17'58"S 50º50'21"W), respectively, and at INMET meteorological stations for Jundiaí (23º31'12"S 46º52'12"W) and Paraíso do Sul (29º43'12"S 53º43'12"W), respectively.

In compliance with the minimum requirements established by the IN SDA 13 and IN SDA 53 for the registration of a microbiological soil conditioner, dry biomass of shoots, grain yield, N and P concentration and accumulation in the shoot biomass were evaluated. For those purposes, 30 plants were collected at the R4-R5 phenological stages from the four central lines of each plot (20 m^2 working area). The biomass was dried in a forced air circulation oven at 60 °C until weight stabilization. Grain yield was determined based on the yield obtained for the 30 plants evaluated per plot. In addition to the above-listed variables, soil enzyme activity was also assessed by analyzing the hydrolysis of Fluorescein Diacetate (FDA), related to overall microbial activity. The enzymatic activity was determined by collecting a sample of 200 grams of soil in each plot. Soil samples were refrigerated and sent to the laboratory for analysis using the methodology described in ALEF & NANNIPIERI (1995).

The concentration and accumulation of N and P were determined from a sample composed of 10 leaves collected in different portions of plants randomly selected in the working area of each plot following the method described by TEDESCO et al. (1995). The leaf closest to the last ear was collected at the time of the appearance of female inflorescences. After the data met the assumptions of homogeneity of variances (Bartlett), a one-way analysis of variance was performed and the separation of means obtained by Tukey's test at 5% probability using the software SISVAR v.5.3.

RESULTS AND DISCUSSION

Crop response to the application of Pseudomonas thivervalensis SC5

The corn crop was highly responsive to the inoculation of *P. thivervalensis* SC5, since practically all parameters (variables) evaluated were positive and statistically significant in all locations. In order to better exemplify these effects, the following aspects related to the parameters evaluated in this study are presented and discussed, namely: shoot biomass production, nutrient concentration and accumulation (N and P) in the tissues, grain yield, as well as the evaluations concerning the hydrolysis of Fluorescein Diacetate (FDA). Table 4 provides a summary of the main results obtained in the evaluations, presenting averages obtained from 10 repetitions per treatment and location.

Figure 1, in turn, presents the average increase observed for each parameter evaluated when compared to the treatment with half the dose of N and non-inoculated (N50-NI). It is important to point out the positive and remarkable effects (in the order of 2 digits for some variables and locations) observed by the use of *P. thivervalensis* SC5 in practically all the places where the experiments were conducted.

Shoot dry biomass production and grain yield

As can be seen in table 4 and figures 1 and 2, the increase in shoot dry biomass (SDB) and grain yield of corn treated with *P. thivervalensis* SC5 was positive (when compared to all other treatments) and statistically significant in all locations where the experiments were conducted.

Pseudomonas thivervalensis SC5 provided average increments in SDB of 10.1% in Mafra (SC), 16.5% in Jundiaí (SP), 30.3% in Canoinhas (SC) and 40.6% in Paraíso do Sul (RS) when compared to the treatment with 50% N and not inoculated (N50-NI) (Figure 1). As for grain yield, these increases were around 10.6% in Mafra (SC), 9.0% in Jundiaí (SP), 27.8% in Canoinhas (SC) and 14.5% in Paraíso do Sul (RS).

Azospirillum brasilense (NITRO 1000) promoted increases in grain yield, compared to treatment N50-NI, of 3.4% in Mafra (SC), 7.7% in Jundiaí (SP), 1.5% in Canoinhas (SC) and 11.0% in Paraíso do Sul (RS). For SDB, the increases promoted by *A. brasilense* were 3.3% in Mafra (SC), 8.3% in Jundiaí (SP), 17.0% in Canoinhas (SC) and 13.3% in Paraíso do Sul (RS), compared to treatment N50-NI. These results are within the range of those reported by other authors for *A. brasilense*. In a meta-analysis conducted by Barbosa et al. (2022), which compiled information from 60 articles published between 2010 and 2021 and 103 field trials in 54 locations in Brazil, they reported average yield increases of 5.4% when the crop was inoculated with *A. brasilense*.

Shoot dry biomass and grain yield were also significantly improved by *P. thivervalensis* SC5 when compared to treatment N100-NI (100% of the recommended N and not inoculated) and N50-NITRO 1000. For SDB, increments varied from 4.7% to 24%. For grain yield, the increments varied from 1.2% to 26%.

These increments are expressive, both for biomass and for grain yield, and are the result of the activity of *P. thivervalensis* SC5 modulating nutrient acquisition mechanisms and adaptations to the environment, which may include resistance to biotic and abiotic stresses, for example. Biomass production and crop yield are, in general, the most important parameters observed when trying to determine the

Table 4 - ANOVA summary for the evaluation of the agronomic efficiency of the microorganism *Pseudomonas thivervalensis* SC5 in Brazil.

Variables	State/Municipality	----F value[1]----	Variation Coefficient (%)	-----Treatments[2]-----			
				N50-NI	N50-SC5	N50-NITRO 1000	N100-NI
Dry biomass (kg ha^{-1})	SC/Mafra	4,76***	5.86	8,404.5 b	9,255.5 a	8,681.7 ab	8,840.7 ab
	SP/Jundiaí	12.98***	6.27	7,312.7 c	8,518.4 a	7,921.7 ab	7,398.1 bc
	SC/Canoinhas	9.98***	11.28	3,606.0 c	4,700.4 a	4,220.0 ab	3,935.7 bc
	RS/Paraíso do Sul	9.63***	14.88	5,135.1 c	7,217.6 a	5,820.5 bc	6,568.2 ab
Grain yield (kg ha^{-1})	SC/Mafra	4.68***	7.87	11,908.3 b	13,171.1 a	12,315.4 ab	13,334.3 a
	SP/Jundiaí	6.02***	4.84	9,532.0 b	10,385.7 a	10,263.1 a	10,025.9 ab
	SC/Canoinhas	20.23***	8.41	4,374.2 b	5,591.5 a	4,437.7 b	5,138.2 a
	RS/Paraíso do Sul	6.46***	7.20	3,146.9 b	3,604.4 a	3,359.2 ab	3,493.7 a
Leaf N concentration (g kg^{-1})	SC/Mafra	8.05***	11.68	5.55 b	6.84 a	5.65 b	5.61 b
	SP/Jundiaí	8.51***	6.16	6.30 b	7.22 a	7.00 a	6.82 a
	SC/Canoinhas	27.99***	6.32	5.98 c	7.31 a	6.56 b	7.38 a
	RS/Paraíso do Sul	7.80***	6.29	6.22 b	7.07 a	6.64 ab	6.94 a
Leaf P concentration (g kg^{-1})	SC/Mafra	5.92***	6.74	0.66 b	0.73 a	0.71 ab	0.75 a
	SP/Jundiaí	7.05***	9.59	0.42 b	0.49 a	0.50 a	0.51 a
	SC/Canoinhas	1.92^{ns}	8.19	0.47 a	0.51 a	0.50 a	0.49 a
	RS/Paraíso do Sul	3.47**	10.01	0.83 b	0.94 a	0.91 ab	0.95 a
Accumulated N (kg ha^{-1})	SC/Mafra	12.07***	13.20	46.69 b	63.32 a	48.90 b	49.65 b
	SP/Jundiaí	21.23***	8.58	46.02 c	61.53 a	55.52 b	50.46 bc
	SC/Canoinhas	21.02***	12.88	21.57 c	34.32 a	27.65 b	29.75 b
	RS/Paraíso do Sul	13.77***	16.92	31.96 c	50.96 a	38.45 bc	45.56 ab
Accumulated P (kg ha^{-1})	SC/Mafra	12.51***	7.69	5.58 c	6.78 a	6.16 bc	6.64 ab
	SP/Jundiaí	11.80***	11.49	3.08 b	4.16 a	3.94 a	3.75 a
	SC/Canoinhas	16.36***	11.34	1.68 c	2.37 a	2.09 ab	1.92 bc
	RS/Paraíso do Sul	21.31***	13.58	4.26 c	6.80 a	5.22 b	6.17 a
FDA (μg Fluorescein g^{-1} dry soil h^{-1})	SC/Mafra	16.26***	5.65	50.08 b	58.33 a	50.43 b	53.39 b
	SP/Jundiaí	76.77***	5.94	24.29 c	34.00 a	24.70 c	26.67 b
	SC/Canoinhas	34.03***	5.96	29.88 c	38.7 a	32.47 b	34.59 b
	RS/Paraíso do Sul	300.63***	3.18	18.96 d	27.93 a	20.46 c	23.44 b

[1]*** = significant at 1% probability; ** = significant at 5% probability; ns = non-significant. Different letters between treatments by location indicate a significant difference at the 5% level of error probability by Tukey's test.

[2]N50-NI = non-inoculated and with 50% of the recommended N; N50-SC5 = inoculated with SC5 and with 50% of the recommended N; N50-NITRO = inoculated with a commercial *Azospirillum brasilense* product (NITRO 1000) and with 50% of the recommended N; N100-NI = non-inoculated and with 100% of the recommended N.

INCREMENT (%)	SC / MAFRA						
TREATMENTS	YIELD	DRY BIOMASS	N CONCENTRATION	P CONCENTRATION	ACCUMULATED N	ACCUMULATED P	FDA
N50-NITRO 1000	3.4	3.3	1.7	6.7	4.7	10.3	0.7
N50-SC5	10.6	10.1	23.1	10.1	35.6	21.4	16.5
N100-NI	12.0	5.2	1.0	13.2	6.3	19.0	6.6

INCREMENT (%)	SP / JUNDIAÍ						
TREATMENTS	YIELD	DRY BIOMASS	N CONCENTRATION	P CONCENTRATION	ACCUMULATED N	ACCUMULATED P	FDA
N50-NITRO 1000	7.7	8.3	11.0	18.2	20.6	28.0	1.7
N50-SC5	9.0	16.5	14.5	16.1	33.7	35.0	40.0
N100-NI	5.2	1.2	8.3	20.5	9.6	21.8	9.8

INCREMENT (%)	SC / CANOINHAS						
TREATMENTS	YIELD	DRY BIOMASS	N CONCENTRATION	P CONCENTRATION	ACCUMULATED N	ACCUMULATED P	FDA
N50-NITRO 1000	1.5	17.0	9.7	7.0	28.2	24.8	8.7
N50-SC5	27.8	30.3	22.1	8.7	59.1	41.4	29.5
N100-NI	17.5	9.1	26.7	5.5	37.9	14.7	15.8

INCREMENT (%)	RS / PARAÍSO DO SUL						
TREATMENTS	YIELD	DRY BIOMASS	N CONCENTRATION	P CONCENTRATION	ACCUMULATED N	ACCUMULATED P	FDA
N50-NITRO 1000	6.7	13.3	6.7	9.2	20.3	22.5	7.9
N50-SC5	14.5	40.6	13.7	13.7	59.5	59.7	47.4
N100-NI	11.0	27.9	11.5	13.8	42.6	44.9	23.7

Figure 1 - Mean increments between treatments when compared to treatment with 50% N and non-inoculated (N50-NI).

effect of a product/management/intervention on the development of a species of agricultural interest. These parameters are important because they directly represent the gains that can be obtained by the farmer when implementing a new activity, a new product, or even a new crop management system.

Pseudomonas thivervalensis SC5 has the ability to express the enzyme 1-aminocyclopropane-

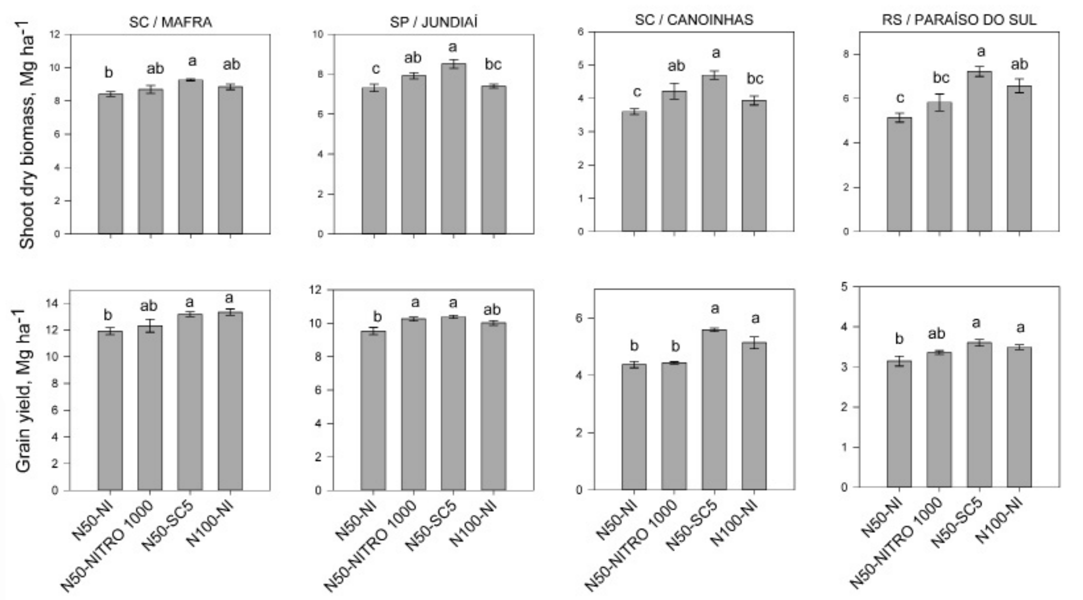

Figure 2 - Effect of *Pseudomonas thivervalensis* SC5 on shoot dry biomass and grain yield of corn. Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5% probability.

1-carboxylate (ACC) deaminase and the subsequent modulation of the plant hormone ethylene through the catabolism of its direct precursor, ACC (SHAHZAD et al., 2013; GLICK, 2014; NAING et al., 2021; HERPELL et al., 2023). Ethylene is one of the most important plant hormones in the regulation of plant growth and development, being involved in multiple physiological and developmental processes of plants (VAN DE POEL et al., 2015; DUBOIS et al., 2018), as well as in the regulation of plant-microorganism interactions (GUINEL, 2015; NASCIMENTO et al., 2018). In addition, ethylene is also involved in plant responses to stress conditions, including those induced by biotic (e.g. pathogens, insects, nematodes) and abiotic (e.g. salinity, low nutrient availability, low pH, heavy metals and organic contaminants) factors (GAMALERO & GLICK, 2015; TAO et al., 2015; KEUNEN et al., 2016). Therefore, bacteria that exhibit ACC deaminase activity decrease deleterious levels of ACC and ethylene induced by biotic and abiotic stresses that inhibit plant growth (BELIMOV et al., 2009; NASCIMENTO et al., 2013; ALI et al., 2014; JAEMSAENG et al., 2018; GUPTA et al., 2022).

The genus *Pseudomonas* and its species that have been proven to promote plant growth, such as *P. thivervalensis* (SHAHZAD et al., 2013; NASCIMENTO et al., 2021), *P. putida* (CHENG et al., 2012; NASCIMENTO et al., 2019) and *P. fluorescens* (SALAMONE et al., 2012; ZERROUK et al., 2016), are capable of producing siderophores (such as pyoverdine – $dPYO_{thi}$ – and histocorrugatin) (MATTHIJS et al., 2016; NASCIMENTO et al., 2021) that are directly associated with the solubilization of Fe in the soil and its availability to plants. These species are also able to synthesize plant growth phytohormones, especially Indole Acetic Acid (IAA), responsible for cell elongation, cell division and differentiation, apical dominance, light response, culminating in direct interferences in plant development (NASCIMENTO et al., 2021).

Laboratory experiments indicated that the inoculation of *P. thivervalensis* SC5 led to a significant increase in the growth of cucumber plants compared to non-inoculated control plants, confirming the plant growth promoting properties of the SC5 strain (NASCIMENTO et al., 2021). When compared to the non-inoculated control treatment, *P. thivervalensis* SC5 increased the dry biomass of cucumber plants by 25%. Under saline conditions, this difference was even greater (44.7%), indicating the strain's potential in conditioning/regulating the plant growth environment. The results showed that plants subjected to saline stress and inoculated with the SC5 strain had similar biomass values to non-inoculated, non-stressed control plants.

These and other characteristics help explain the fact that *P. thivervalensis* SC5 (N50-SC5) provided higher SDB and grain yield, not only in relation to the N50-NI treatment, but also, in most cases, to the other treatments tested. For both SDB and grain yield, it is possible to verify that *P. thivervalensis* SC5 was superior (in most cases) or equal (statistically) to treatments N100-NI and N50-NITRO 1000, the latter containing the recommended strains of *Azospirillum brasilense* AbV5 and AbV6.

Concentration and accumulation of N and P in plant biomass

As can be seen in table 4 and figures 1 and 3, when compared to all other treatments, treatment N50-SC5 showed significant increases in the levels and in the accumulated values of N and P in the tissues of corn in all the evaluated places, with the exception of the P concentration for the municipality of Canoinhas (SC). With the exception of Paraíso do Sul (PR) for the variable P concentration (significant at 5% probability), the results of the 4 variables were significant at 1% probability level for all locations (Table 4).

When compared to the treatment with 50% of N and not inoculated (N50-NI) (Figure 1), *P. thivervalensis* SC5 provided average increments of N and P concentration in the tissues of 23.1% (N) and 10.1% (P) in Mafra (SC), 14.5% (N) and 16.1% (P) in Jundiaí (SP), 22.1% (N) and 8.7% (P) in Canoinhas (SC), and 13.7% (N and P) in Paraíso do Sul (RS). As for the accumulation of these nutrients in the tissues, the results showed increments of 35.6% (N) and 21.4% (P) in Mafra (SC), 33.7% (N) and 35% (P) in Jundiaí (SP), 59.1% (N) and 41.4% (P) in Canoinhas (SC), and 59.5% (N) and 59.7% (P) in Paraíso do Sul (RS).

These increments are significant and above all other treatments, both for the concentration and accumulation of those nutrients, and directly suggest that *P. thivervalensis* SC5 acts on mechanisms of soil modulation and acquisition (solubilization and mineralization) of nutrients, a trait well-known for this isolate (NASCIMENTO et al., 2021). *Pseudomonas thivervalensis* SC5 has the ability to solubilize PO_4 and ZnO. In addition, it synthesizes the IAA hormone, reduces nitrate to N_2 and ammonia, which is consistent with its ability to use KNO_3 and KNO_2 as the sole nitrogen sources (NASCIMENTO et al., 2021). *Pseudomonas thivervalensis* SC5 reduces nitrate to produce N (gas) and ammonia, indicating that the

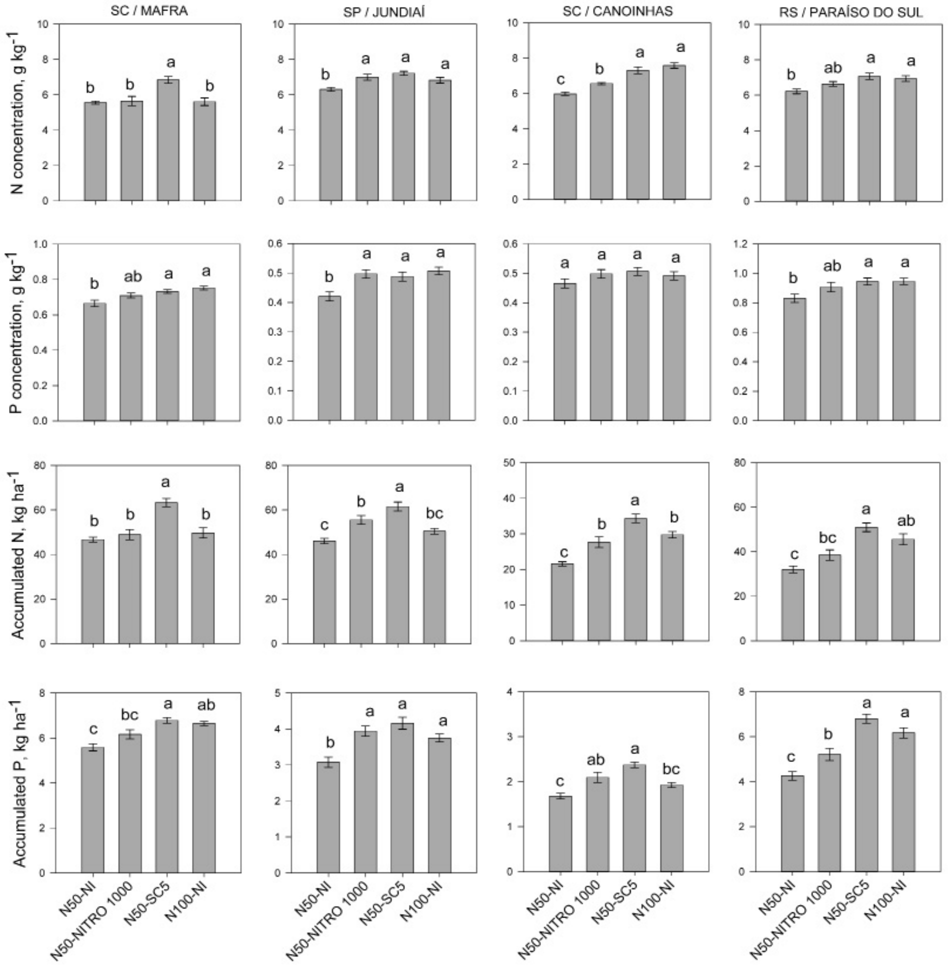

Figure 3 - Effect of *Pseudomonas thivervalensis* SC5 on the concentration and accumulation of N and P in the tissues of corn. Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5% probability.

dissimilatory nitrate reduction and denitrification pathways are active in this strain. These results are consistent with the presence of genes responsible for the dissimilatory pathways of nitrate reduction (*nas*) and denitrification (*nir*, *nar*, *nor*), as well as several genes involved in the transport of nitrate, nitrite and ammonia (three *amtB* genes) present in the strain's genome (NASCIMENTO et al., 2021). In addition, genes involved in the transport and degradation of urea and allophanate were also reported. These genes are contained in the *ureABC* cluster responsible for the production of urease and *atzF* that encodes allophanate hydrolase.

The genome of strain SC5 contains the pyrroloquinoline quinone (pqq) operon and glucose dehydrogenase genes involved in the production of gluconate, one of the main organic acids involved in the solubilization of mineral phosphate and other compounds such as ZnO. In addition, genes encoding enzymes involved in the solubilization of organic phosphate, such as an extracellular phytase, several alkaline phosphatases, and some components of the phosphonate C-P lyase system were also found in the genome of the SC5 (NASCIMENTO et al., 2021). The extracellular phytase identified in the genome of the strain SC5 (CE140_04710) bears some resemblance to the extracellular 3-phytase produced by *Bacillus subtilis*, which is involved in the catabolism of phytate, a common plant metabolite found in plant tissues and soils, which serves as a source storage of phosphorus and inositol (ZENG et al., 2011).

Two copies of the genes encoding the phosphate transport system (pstABCS) were also detected in the genome of *P. thivervalensis* SC5 (NASCIMENTO et al., 2021). In general, the data obtained indicate that *P. thivervalensis* SC5 participates in the N, P and S cycles through different enzymatic activities, thus increasing the availability of several nutrients essential for plant growth.

Soil enzymatic activity by hydrolysis of fluorescein diacetate (DAF)

As can be seen in table 4 and figures 1 and 4, the increase in the hydrolysis of FDA in the soil treated with *P. thivervalensis* SC5 was positive (when compared to all other treatments) and statistically significant in all locations. As evidenced by table 4, the results for this variable were significant at 1% probability level for all locations.

When compared to the treatment with 50% N and not inoculated (N50-NI) (Figure 1), *P. thivervalensis* SC5 provided average increments in the hydrolysis of FDA in the order of 16.7% in Mafra (SC), 40.0% in Jundiaí (SP), 29.5% in Canoinhas (SC), and 47.4% in Paraíso do Sul (RS).

Soil conditioning promoted by *P. thivervalensis* SC5, evaluated through the hydrolysis of FDA, confirms the ability of the strain to act in nutrient cycling, increasing the supply of nutrients to the crop. The analysis of the hydrolysis of Fluorescein Diacetate (FDA) indicates the activity of a group of enzymes present in the soil, which include lipases, esterases and proteases (BALOTA et al., 2013), responsible for facilitating the degradation of organic matter, releasing essential nutrients to plants (PROSSER et al., 2011).

The soil enzymatic analysis, capable of representing the microbiological activity of the soil, has greater sensitivity than chemical and physical analyses, being able to detect subtle changes in soil properties that occur in a short period of time, after

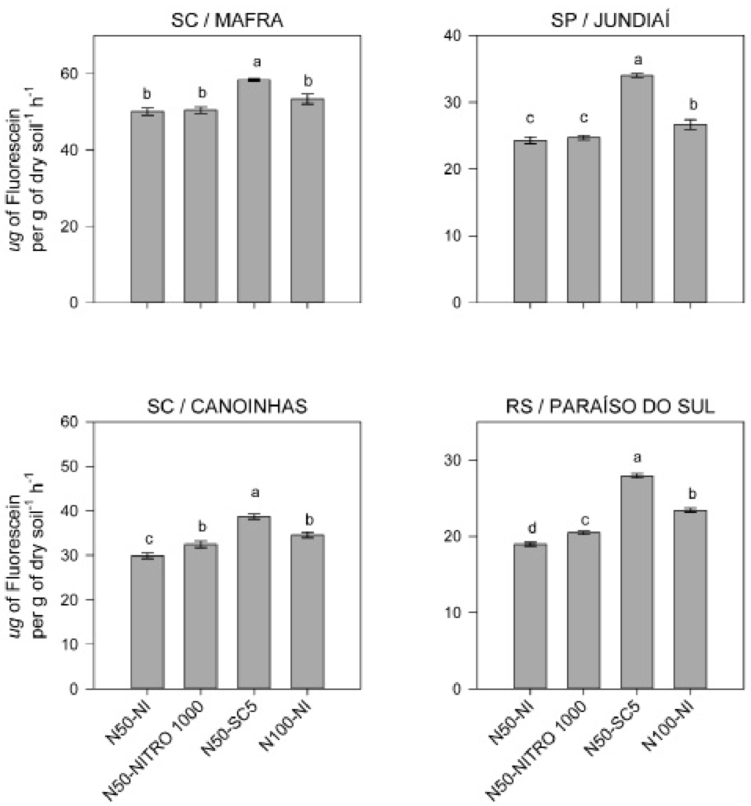

Figure 4 - Effect of *Pseudomonas thivervalensis* SC5 on the hydrolysis of Fluorescein Diacetate (FDA). Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5% probability.

the introduction of different practices or systems of agricultural management (KNUPP et al., 2010; STOTT et al., 2010; BALOTA et al., 2013; BILEN & TURAN, 2022). Thus, the microbiological and biochemical attributes are excellent bioindicators of soil quality and/or the sustainability of the soil-plant system (DINESH et al., 2003; NOGUEIRA et al., 2006), and can be used to evaluate the efficiency of conditioners to improve important characteristics linked to plant development.

Bacteria from the genus *Pseudomonas* are widely known as plant growth promoters, acting directly or indirectly on plant development. In a study carried out by SANDHYA et al. (2010), testing the inoculation of different *Pseudomonas* strains (*P. entomophila* strain BV-P13, *P. stutzeri* strain GRFHAP-P14, *P. putida* strain GAP-P45, *P. siringae* strain GRFHYTP52 and *P. monteilli* strain WAPP53) in soil with water stress, it was found that all strains were able to improve plant development. In the study of those authors, inoculation of *Pseudomonas* promoted increases in plant root and shoot biomass, as well as increases in the contents of amino acids, proline, soluble sugars, proteins and water in the leaves. In that study, the authors verified that the inoculated treatments were able to reduce the activity of the antioxidant enzymes APX, CAT and GPX, indicating an effect of reducing the stress caused by water deficit. In addition, the inoculation also promoted an increase in the stability and average diameter of aggregates in the soil, that is, it promoted soil conditioning.

In another study, involving the co-inoculation of *Pseudomonas* and *Bradyrhizobium japonicum* in soybean, the results showed that the presence of the *Pseudomonas* strain ensured significant increases in the number and mass of plant nodules, as well as provided greater increments in the growth and uptake of N and P by plants (ARGAW, 2012). As with soybean, beans also benefit from the co-inoculation of *Pseudomonas* (*P. fluorescens* and *P. monteilii*) together with strains of *Rhizobium*, increasing the number of nodules and the biological nitrogen fixation, resulting in higher dry matter and grain yield (YADEGARI et al., 2010; SÁNCHEZ et al., 2014).

The SC5 strain has been shown to have a complete degradation pathway for hydroxycinnamic acids. The ability to use hydroxycinnamic acids as a carbon source indicates that *P. thivervalensis* SC5 may also play an important role in rhizospheric colonization activities, as well as in maintaining soil and plant health, decreasing the long-term accumulation of these allelochemicals in the system (BATISH et al., 2008; SINGH et al., 2014; FERRO et al., 2015).

CONCLUSION

Pseudomonas thivervalensis SC5 has different mechanisms of action in the soil-plant system. These mechanisms bring improvements such as increased dry matter production, grain yield, absorption and accumulation of nutrients (N, P, S, etc.), among other characteristics. *Pseudomonas thivervalensis* SC5 is able to modulate important biochemical pathways that regulate processes responsible for the mineralization/solubilization of chemical agents that are fundamental for the improvement of soil quality, which leads, in this case, to a differentiated conditioning of the soil-plant system, a result that it is unspecific, that is, it does not depend on a particular crop to which the strain is associated.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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