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Effects of plant growth-promoting bacteria on the performance of chickpea seedlings

Ruan da Silva CÂNDIDO[1], Patrícia Costa SILVA[1], Josué Gomes DELMOND[1], Pedro Rogerio GIONGO[1], Eduardo Silva COUTO[1], Lucas Inácio da SILVA[1], Ana Flávia Alves FERREIRA[1], Emanuelly Silva ARAÚJO[1], Carlos Miguel Barbosa VALÉRIO[1], Adriana Rodolfo da COSTA[1*]

Abstract

Using plant growth-promoting bacteria (PGPB) is a sustainable option for the cultivation of chickpeas as well as other crops, and their effects can be observed from the beginning of development. The objective of this study was to evaluate the development of the seedlings of chickpea cultivars under inoculation with PGPB. The experiment was conducted in a greenhouse, and the experimental design adopted was completely randomized in a 2 × 3 factorial scheme (2: application or not of the inoculant in chickpea seed treatment; 3 chickpea cultivars: BRS Aleppo, BRS Cícero, and BRS Toro). In this research, it was observed that growth-promoting bacteria showed positive effects in the first days of chickpea development, having a more significant effect on the growth and accumulation of root mass. The authors suggested that biomass accumulation in the root system is due to the secretion of substances by microorganisms inoculated via seeds, which promote root development and consequently greater absorption of nutrients. The BRS Aleppo cultivar showed better performance in terms of seedling emergence, but the seedlings had lower biomass accumulation in both shoots and roots. The use of PGPB promoted fast and early chickpea growth.

Keywords: *Cicer arietinum* L.; *Rhizobacteria*; bioinputs; root mass accumulation.

Practical Application: Inoculation of plant growth-promoting bacteria in the seeds promoted fast and early chickpea growth.

1 INTRODUCTION

Chickpeas (*Cicer arietinum* L.) are the third-most cultivated legumes in the world, only behind peas and beans (Romanyà & Casals, 2020), and have social, environmental, and nutritional importance (Palmero et al., 2022). Chickpea seeds are rich in nutrients such as calcium, magnesium, phosphorus, potassium, zinc, iron, sulfur, and folic acid, besides containing several vitamins (Chang et al., 2022). In this context, it is also considered an important legume for ensuring food and nutritional security (Kaur & Prasad, 2021).

Chickpea cultivation has expanded in Brazil, mainly in areas belonging to the Cerrado biome (Palmero et al., 2022). This is due to the intensification of research and the availability of cultivars from the *Kabuli* group, adapted to the edaphoclimatic variations of the region, among which BRS Aleppo, BRS Cícero, and BRS Toro stand out as the most productive, with an average yield of 2,500 kg ha^{-1} (Nascimento & Silva, 2019).

In recent years, there has been a growing adoption of more sustainable systems that aim to increase agricultural yield, with less impact on the environment (Santos et al., 2019). One of the first steps toward profitable production is adequate knowledge about aspects related to the availability of high-quality seeds. In addition, to obtain higher yields, there must be a greater demand for nutrients by the crops, especially nitrogen and phosphorus, as well as the production of phytohormones that synthesize growth regulators, which are related to biomass and grain production (Joshi et al., 2019; Zhang et al., 2020). The increased use of bioinputs in various crops is noticeable, including the use of plant growth-promoting *Rhizobacteria*, which is also a sustainable option in chickpea cultivation (Sharma et al., 2019; Verma et al., 2020).

In this context, the use of plant growth-promoting bacteria (PGPB) of the species *Rhizobium tropici*, *Azospirillum brasilense*, *Pseudomonas fluorescens,* and *Saccharomyces* sp. is feasible from an economic and sustainable point of view because they are capable of synthesizing phytohormones such as auxins, indole acetic acid, 1-aminocyclopropane-1-carboxylatedeaminase, cytokinin, gibberellin and bioactive elements, such as enzymes (Balbinot et al., 2020). Furthermore, these hormones cause changes in the root morphology, which results in a greater absorption of nutrients and water (Mukherjee et al., 2020). Bacteria also act in biological nitrogen fixation and phosphorus solubilization, induce systemic resistance, and assist in biocontrol against phytopathogens (Cassán et al., 2020).

Therefore, knowledge about aspects related to the quality of the seeds of chickpea cultivars is important to ensure profitable

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[1]*Universidade Estadual de Goiás, Campus Sudoeste, Unidade Universitária de Santa Helena de Goiás, Departamento de Engenharia Agrícola, Santa Helena de Goiás, GO, Brazil.*
*Corresponding author: adriana.costa@ueg.br
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and sustainable production, especially regarding the initial establishment of the crop in the field and the association with efficient bacteria. Given these considerations, the objective of this study was to evaluate the development of the seedlings of chickpea cultivars under inoculation with PGPB.

2 MATERIAL AND METHODS

2.1 Experimental area characterization

The experiment was conducted in the experimental area of the Universidade Estadual de Goiás, University Unit of Santa Helena de Goiás, located in the southwest of the Goiás state, Brazil (17°49'34.3" South, 50°36'24.4" West, and 570 m altitude), as shown in Figure 1. According to Köppen's classification, the climate in the region is Aw, tropical with a dry winter (Alvares et al., 2013) and well-defined precipitation in two seasons of the year: a rainy season known as summer, from November to April, and a dry season known as winter, from May to October. Based on climate normals from the National Institute of Meteorology (INMET), Silva et al. (2022) estimated the average temperature for the location to be 23°C, while the minimum is 17.6°C and the maximum is 29.8°C, with an average annual precipitation of 1,612.90 mm.

2.2 Experimental design

The experimental design used was completely randomized, in a 2 × 3 factorial scheme (2: application or not of the inoculant and 3: chickpea cultivars--BRS Aleppo, BRS Cícero, and BRS Toro), with six treatments and six replicates with 40 seeds, to make up 240 seeds per treatment, as recommended by the Standards for Seed Analysis (Brasil, 2009), totaling 36 plots.

2.3 Experimental setup, inoculation, and irrigation

The experiment was set up in a protected environment (greenhouse). The soil used in the experiment was a *Latossolo Vermelho Distrófico* (Oxisol) with a clayey texture, typical of the region (Santos et al., 2018), whose chemical and textural analysis is presented in Table 1.

Each plot consisted of pots with a capacity of 15 L. These pots were filled with soil sieved through a 1-mm-mesh sieve, which was not corrected or fertilized to avoid possible interference with the inoculant containing PGPB in the soil. Sowing was carried out manually on September 30, 2022. Two concentric circles were arranged according to the dimensions of the pot, with 26 seeds in the outer circle (24 cm in diameter) and 14 seeds in the inner circle (20 cm in diameter), distributed equidistantly every 3 cm and at a depth of 3 cm, with a spacing of 50 cm between rows/pots. Phytosanitary control was not necessary.

The inoculant used was BioStart, manufactured by Biosphera Agro Solutions, which is composed of a combination of bacteria from the genera *Pseudomonas*, *Azospirillum*, *Saccharomyces,* and *Rhizobium*. The dose recommended by the manufacturer for applying this inoculant is 100-150 mL 10 m^{-3}, and the highest dose was chosen to be applied in treatments related to inoculation in this experiment. Finally, the proportional dose was diluted in the irrigation water, whose volume was calculated based on the reference evapotranspiration (E_{To}) of the day, and applied individually and uniformly in each pot.

Data on the maximum and minimum temperatures and relative humidity, as well as water evaporation from a mini pan evaporimeter (MPE) installed inside the greenhouse, were collected daily. The irrigation applied was determined based on E_{To}, with a daily irrigation interval.

E_{To} was established based on the amount of water evaporated from the MPE, made of polyvinyl chloride, according to Santos et al. (2017). The calibration equation (Equation 1) was used to determine the evaporation from the standard Class A pan, which was then multiplied by the pan coefficient (k_p = 1.0) to obtain E_{To} (mm day^{-1}), according to Equation 2 (Allen et al., 2006). Based on the pot area, the irrigation depth was converted to volume, and irrigation was carried out uniformly throughout the entire area of the pot every day using a graduated cylinder and a mini watering can:

$$ECA = 1.4035\,(MPE) + 1.2456 \tag{1}$$

Where:

ECA: Evaporation from the Class A pan (mm day^{-1});

MPE: Evaporation from the mini pan evaporimeter (mm day^{-1}):

$$E_{To} = k_p \times ECA \tag{2}$$

Table 1. Chemical and textural analysis of the soil used in the experiment.

pH	P mlch-1		$S\_SO_4^{-2}$		Ca^{2+}	K^{+}	Mg^{2+}	Al^{3+}	H+Al	SB		t	T
$CaCl_2$		mg dm^{-3}					cmol$_c$ dm^{-3}						%
5.30	6.40		2.80		1.75	0.04	0.27	0.00	2.15	2.06		2.06	4.22
OM	V	m		B	Cu	Fe	Mn	Zn	Na		Sand	Silt	Clay
g dm^{-3}	%					mg dm^{-3}						g kg^{-1}	
14.20	48.93	0.00		0.33	2.80	35.20	16.03	0.70	1.0		445	150	405

pH: hydrogen potential ($CaCl_2$), P mlch-1: phosphorus (Mehlich-1); K^+: potassium; $S\_SO_4^{-2}$: sulfur sulfate; Ca^{2+}: calcium; Mg^{2+}: magnesium; Al^{3+}: aluminum; H+Al: hydrogen plus aluminum; SB: sum of bases; OM: organic matter; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; Na: sodium; T: potential cation exchange capacity; t: effective cation exchange capacity; V: base saturation; m: aluminum saturation.

Where:

E_{To}: Reference evapotranspiration (mm day^{-1});

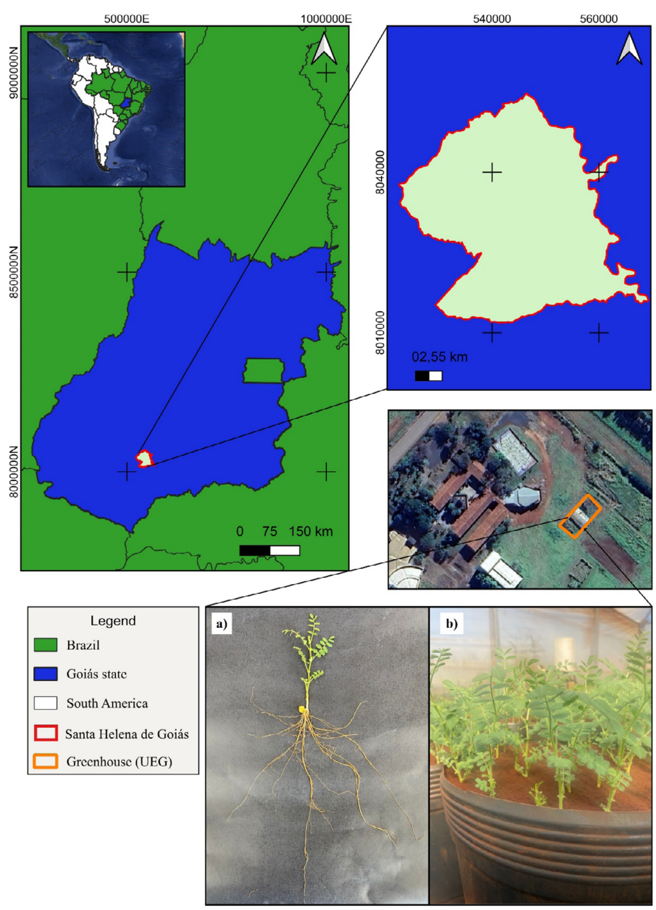
k_p: Pan coefficient (dimensionless);

ECA: Pan evaporation (mm day^{-1}).

2.4 Seedling analysis

The protocol was carried out in accordance with the relevant guidelines of "Rules for Seed Analysis" (RSA) (Brasil, 2009). To evaluate the effect of the inoculation of phytohormone-synthesizing and PGPB on the chickpea cultivars in the seedling emergence stage (Figures 1A and 1B), which lasted 13 days, the following parameters were determined:

- Emergence speed (ES): ES was determined by the arithmetic mean of the values of seeds that emerged during the treatment, obtained for the six replicates of 40 seeds;
- Emergence speed index (ESI): ESI was determined along with the seedling emergence test, by recording daily, at the same time, the number of seedlings that had a visible hypocotyl loop, until stabilization, according to the equation proposed by Magruire (1962) (Equation 3):



UEG: Universidade Estadual de Goiás.

Figure .1 Location of the study area, and the stabilization of chickpea seedlings' emergence after their removal for (A) evaluation and (B) in the pot.

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \cdots + \frac{EN}{Nn} \quad (3)$$

Where:

ESI: Emergence speed index;

E1, *E2*, ... *En*: Number of normal seedlings (NS) emerged on the first day, on the second day, and so on until the last count (n), up to stabilization, which occurred 13 days after emergence;

N1, *N2*, ... *Nn*: Number of days from sowing to the first count, to the second count, and so on until the last count (n), up to stabilization.

- Emergence speed coefficient (ESC): This coefficient was obtained by the arithmetic mean of the total seedlings (TS) counted minus the number of NS from the previous day. According to Equation 4, the higher the result, the greater the germination speed, making the lot more vigorous (Roos and Moore III, 1975):

$$ESC = \frac{E1 + E2 + E3 \ldots En}{N1E1 + N2E2 + N3E3 \ldots NnEn} \quad (4)$$

Where:

ESC: Emergence speed coefficient;

E1, *E2*, ... *En*: Number of well-developed NS according to RAS, emerged in the first count, in the second count, and so on until the last count (n), up to stabilization;

N1, *N2*, ... *Nn*: Number of days from sowing to the first count, to the second count, and so on until the last count (n), up to stabilization;

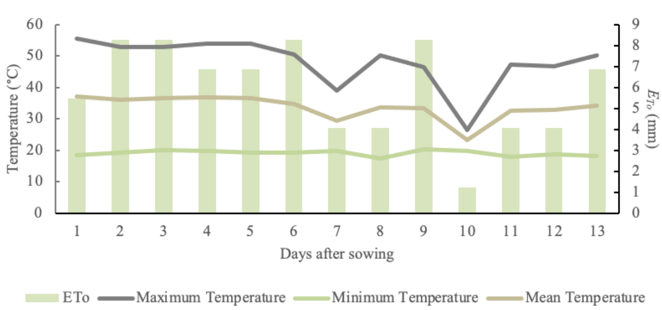
- Emergence (E): This was obtained by the number of NS emerged, determined upon the stabilization of emerged seedlings, with the results expressed as a percentage (%);
- Shoot length (SL) and root length (RL): For obtaining SL and RL, after stabilization of emergence, the average lengths of the shoots and roots of seedlings considered normal were measured using a millimeter ruler, with the results expressed in centimeters (cm) per seedling;
- Stem diameter (SD): SD was determined using a caliper, measured at 2 cm from the ground and expressed in mm;
- Number of leaves (NL): NL was obtained by counting the number of leaves of developed seedlings;
- Shoot fresh mass (SFM), shoot dry mass (SDM), root fresh mass (RFM), and root dry mass (RDM): Twenty NS obtained in the emergence test were weighed to obtain SFM and RFM. Then, the seedlings from each replicate were placed in paper bags and dried in an oven with forced air circulation, at a constant temperature of 65°C, for 72 h. After this period, they were weighed on a precision scale to determine SDM and RDM, and the results were expressed in g seedling^{-1}, according to Nakagawa (1999).

2.5 Statistical analysis

The data obtained for each variable were tested for normality using the Shapiro-Wilk method and for homogeneity of variances using the Levene test, in the statistical program PAST (Hammer et al., 2001). After confirming the assumptions, an analysis of variance associated with the F test was performed at a probability level of 5% ($p < 0.05$), and the Tukey test was applied ($p < 0.05$). This test aimed to find out whether there are significant differences between the means of the treatment factors (cultivars and inoculation of phytohormone-synthesizing and PGPB) and their interactions for each variable, using the statistical program SISVAR (Ferreira, 2019). Additionally, the relationship between variables related to the development of chickpea seedlings was studied using Pearson's correlation analysis at a 5% probability level ($p < 0.05$), using the statistical program PAST (Hammer et al., 2001).

3 RESULTS AND DISCUSSION

Figure 2 presents the climatic data obtained inside the greenhouse during the experimental period. The minimum temperature varied between 17.4 and 20.2°C, and the maximum temperature ranged between 26.5 and 55.7°C. During the first week, the average temperature was higher, 36.6°C, which resulted in greater water evaporation and consequently greater E_{To}, whose value was 8.26 mm day^{-1}; however, when temperatures were low, E_{To} varied between 1.26 and 4.05 mm day^{-1}. During the experiment, 76.54 mm of water was used to irrigate the chickpea cultivars inside the greenhouse.



E_{To}: reference evapotranspiration; PGPB: plant growth-promoting bacteria.

Figure 2. E_{To}, and maximum, mean, and minimum daily air temperatures during the emergence evaluation of the seedlings of the chickpea cultivars inoculated with phytohormone-synthesizing and PGPB.

According to Pendergast et al. (2019), for good chickpea development, the ideal daytime temperature should be between 21 and 30°C, and the nighttime temperature should be close to 20°C. Nascimento et al. (2016) indicated that temperatures between 20 and 30°C are optimal for seed germination, promoting the emergence of seedlings between 5 and 6 days after sowing. However, it was observed that the hypocotyl loops began to appear 3 days after sowing, under the given conditions. In the present study, the maximum temperature inside the greenhouse during some periods of the day reached 55.7°C, but there was no negative influence on seed germination or the development of the seedlings of the chickpea cultivars. According to Araújo and Souza (2018), the success in the use of seeds depends on the occurrence of rapid and uniform germination, followed by the satisfactory establishment of seedlings in the field, as the longer they remain in the initial stages of development, the more vulnerable they will be to adverse conditions in the environment.

Regarding the effect of chickpea cultivars, it can be seen in Table 2 that BRS Aleppo differed from BRS Cícero and BRS Toro for TS, NS, and abnormal seedlings (AS). For AS, the cultivar BRS Aleppo stood out from the others, with 7.33 AS in the pot. According to Hosken et al. (2017), AS are those that do not have good potential to develop a normal plant under favorable field conditions. TS were equal to 27.17 for the BRS Aleppo cultivar, of which 19.83 were normal, which corresponded to an emergence rate of 67.92%. Of this total of seedlings, 12.83 were considered to be hard or dead seeds. BRS Toro and BRS Cícero had lower emergence rates, 46.04 and 37.29%, respectively. Rosa et al. (2021), while testing different substrates for the germination of chickpea cultivars, observed that there was a variation between 15 and 78% when the seeds germinated on a paper roll and between 9% and 56% when the seeds germinated in sand, which demonstrated the variability of this parameter for chickpea seeds.

Pinheiro (2021) evaluated the field emergence of different chickpea cultivars and observed variation in the field emergence from 37.33 to 57.33%, with 37.33% for BRS Aleppo and 52% for BRS Toro. According to the same author, chickpea seeds can have associated fungi from the field, which is why there is great variability in the emergence of different seed lots. According to Trancoso et al. (2021), the chickpea seed coat has a low content or absence of lignin and a high concentration of pectin, which, combined with the high protein content in its constitution, favors the development of fungi. Hosken et al. (2017), while evaluating the physiological

Table 2. Seedling emergence of chickpea cultivars grown under the irrigation and inoculation of phytohormone-synthesizing and plant growth-promoting bacteria.

Cultivars	ES (days)	ESI	ESC	E (%)	TS	NS	AS	NES
BRS Aleppo	6.21^{a}	4.36^{a}	15.49^{a}	67.92^{a}	27.17^{a}	19.83^{a}	7.33^{a}	12.83^{b}
BRS Cícero	5.78^{a}	2.07^{b}	13.47^{b}	37.29^{b}	14.92^{b}	12.58^{b}	2.33^{b}	25.08^{a}
BRS Toro	6.34^{a}	2.64^{b}	13.94^{b}	46.04^{b}	18.42^{b}	15.08^{b}	3.33^{b}	21.58^{a}
LSD	0.84	0.66	1.23	11.05	4.42	2.99	2.47	
CV (%)	13.66	21.48	8.58	21.77	21.77	18.82	16.58	

LSD: least significant difference; CV: coefficient of variation; ES: emergence speed; ESI: emergence speed index; ESC: emergence speed coefficient; E: emergence; TS: total seedlings; NS: normal seedlings; AS: abnormal seedlings; NES: non-emerged seedlings; PGPB: plant growth-promoting bacteria. Means followed by the same letter in the column do not differ from each other by Tukey's test at a 5% probability level.

and sanitary qualities of the seeds of BRS Cícero chickpea grown at different times and under irrigation, detected a high incidence of fungi *Alternaria* sp. (86%). Associated with this, Hosseini et al. (2009) and Parwada et al. (2022) indicated a reduction and delay in chickpea emergence in soils with a more acidic pH.

For ESI, a similar behavior was observed in terms of emergence percentage while comparing the chickpea cultivars, with a variation between 2.07 for BRS Cícero and 4.36 for BRS Aleppo. Dias et al. (2019) also observed lower physiological quality when compared to other cultivars, such as BRS Aleppo, corroborating what was found in the present study. ESI is used to differentiate seed lots in terms of the speed of occurrence of the emergence process, being more sensitive in the differentiation between chickpea seed lots (Castilho et al., 2019; Rosa et al., 2021). According to Peske et al. (2012), this method is based on the principle that lots with the highest ESI are the most vigorous, hence indicating that there is a direct relationship between the seedling formation speed and seed vigor, so seed deterioration reduces the speed of emergence, allowing it to be expressed by ESI (Matthews et al., 2010).

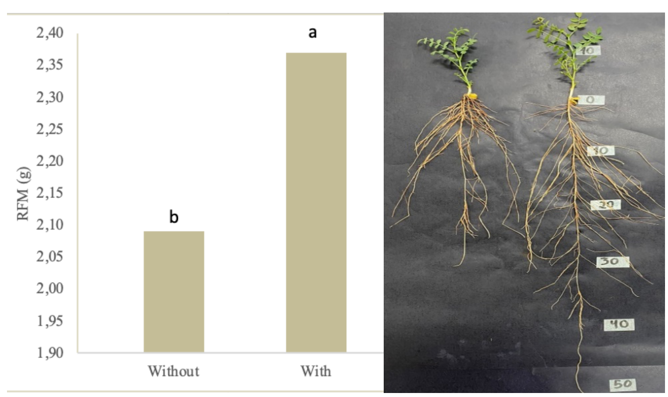
At 13 days after sowing, the chickpea seedlings already had 5-6 leaves on the main branch (Table 3), which, according to the phenological description by Carvalho et al. (2021), would be between vegetative stages V4 and V5. According to these authors, the NL and branches is variable for each cultivar. BRS Aleppo differed from BRS Toro in terms of NL in the initial stage of development. Regarding SL, BRS Aleppo and BRS Cícero stood out from BRS Toro, while SFM accumulation and RDM accumulation were higher in BRS Cícero; these results are probably due to genotypic differences between the tested cultivars. Hosseini et al. (2009) attributed the differences in chickpea RL and SL to the genotype, that is, to the different cultivars. According to Pedó et al. (2014), the reduction in shoot growth affects the distribution of fresh mass intended for the formation of new leaves, which affects the formation of the plant's photosynthetic apparatus, while the reduced growth of the root system in turn results in the less absorption of water and nutrients.

In Figure 3, it is observed that the RFM of the chickpea seedlings was sensitive to the use of PGPB. Figure 3 also shows that the inoculation promoted an 11.81% increase in RFM accumulation in chickpeas still at the seedling stage. This result was due to the synthesis of the phytohormones such as auxin, cytokinins, and gibberellins promoted by bacteria, which promote cell elongation and expansion, resulting in the growth of roots and stems. According to Verma et al. (2020), the inoculation of chickpeas with a combination of PGPB increases root proliferation and consequently leads to the greater absorption of water and nutrients.

Studies conducted in Iran by Maleki et al. (2014) prove that the use of inoculants in seed treatment has positive results and is of great importance for chickpea cultivation. Verma et al. (2020) suggested that biomass accumulation in the root system is due to the secretion of substances by microorganisms inoculated by way of seeds, which promote root development and consequently greater absorption of nutrients.

Almeida Neta et al. (2021) recommended inoculating chickpea seeds with a mixture of *Bacillus* spp. in soil cultivated for a long period with crops, as this practice represents an increase of up to 4% in yield. Xavier et al. (2023) also reported that inoculation with PGPB is a promising strategy for chickpeas, as exudates expelled by their roots promote colonization by PGPB, stimulate biological processes related to nodulation, and can improve chickpea production under field conditions.

Co-inoculation of PGPB is a strategy recommended by several authors for chickpeas, like Abd-Alla et al. (2019) and Zaheer et al. (2019). These authors have demonstrated positive results in root development at as early as the seedling stage, as found in the present study, because PGPB act in the synthesis of growth hormones and secondary metabolites. In this context, although there



LSD: 0.23 g; CV: 15.12%. RFM: root fresh mass; PGPB: plant growth-promoting bacteria; LSD: least significant difference; CV: coefficient of variation; [a,b]Column followed by the same letter do not differ from each other by Tukey test at 5% probability level.

Figure 3. RFM of chickpea cultivars subjected (With) or not (Without) to inoculation with the phytohormone-synthesizing and PGPB.

Table 3. Initial characterization of chickpea cultivars by seedlings grown under inoculation of phytohormone-synthesizing and plant growth--promoting bacteria.

Cultivars	NL	SD	SL	RL	SFM	SDM	RFM	RDM
		(mm)	(cm)	(cm)	(g)	(g)	(g)	(g)
BRS Aleppo	6.70[a]	2.36[b]	12.39[a]	33.97[a]	0.90[b]	0.27[b]	2.31[a]	0.28[b]
BRS Cícero	6.38[ab]	2.78[a]	12.25[a]	35.39[a]	1.08[a]	0.31[ab]	2.34[a]	0.39[a]
BRS Toro	5.66[b]	2.34[b]	11.32[b]	33.86[a]	0.84[b]	0.37[a]	2.04[a]	0.34[ab]
LSD	0.77	0.16	0.89	4.68	0.14	0.058	0.34	0.067
CV (%)	12.33	6.6	10.94	13.51	14.56	18.09	15.12	19.84

LSD: least significant difference; CV: coefficient of variation; NL: number of leaves; SD: stem diameter; SL: shoot length; RL: root length; SFM: shoot fresh mass; SDM: shoot dry mass; RFM: root fresh mass; RDM: root dry mass; PGPB: plant growth-promoting bacteria. Means followed by the same letter in the column do not differ from each other by Tukey's test at a 5% probability level.

was no increase in the emergence of seedlings grown from seeds inoculated with PGPB, a positive effect on root development was observed, which could promote greater vigor in chickpea seedlings.

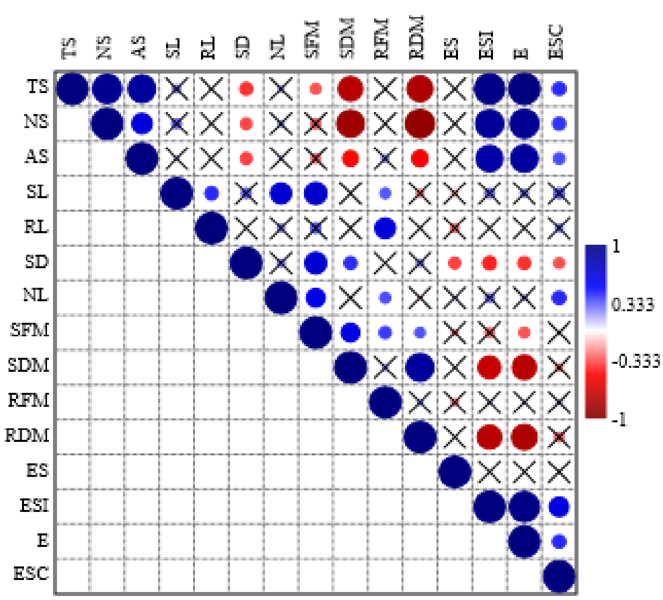
Figure 4 presents Pearson's correlations between variables related to the development of chickpea seedlings. A strong and positive correlation is observed between ESI and the total number of seedlings (r = 0.96) and the total number of NS (r = 0.89), which is confirmed by Faria et al. (2021), that ESI is directly related to the development of NS. However, ESI showed a negative correlation with the dry mass accumulation in both the shoots (r = -0.72) and roots (r = -0.77) of the chickpea seedlings, contrasting with the results observed by other authors, such as mung beans (Faria et al., 2021) and common beans (Amaro et al., 2015).

This highlights the proposition that the fresh and dry mass accumulation in both shoots and roots is related to the genotype since the cultivar that had the best emergence rates, BRS Aleppo (Table 2), showed a smaller SD and lower accumulation of SFM and RDM (Table 3). Freire et al. (2018) observed different behaviors between rice cultivars under irrigation with saline water; one of the cultivars showed greater tolerance to stress related to ES, and the other cultivar showed tolerance related to dry mass accumulation in shoots and roots.

4 CONCLUSIONS

The BRS Aleppo cultivar showed better performance in terms of seedling emergence, but the seedlings had less biomass accumulation in both shoots and roots.

The use of plant growth-promoting bacteria promoted fast and early chickpea growth.



TS: total seedlings; NS: normal seedlings; AS: abnormal seedlings; SL: shoot length; RL: root length; SD: stem diameter; NL: number of leaves; SFM: shoot fresh mass; SDM: shoot dry mass; RFM: root fresh mass; RDM: root dry mass; ES: emergence speed; ESI: emergence speed index; E: emergence; ESC: emergence speed coefficient.

Figure 4. Pearson's correlation between variables related to the initial development of seedlings of chickpea cultivars. "X" indicates a non--significant correlation at a 5% probability level between the variables.

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