

Scoping review: use of biofertilizers and humic substances in *Phaseolus vulgaris* (Fabaceae) for dry zones

Scoping review: uso de biofertilizantes y sustancias húmicas en *Phaseolus vulgaris* (Fabaceae) para zonas secas



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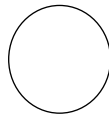
Phaseolus vulgaris pods genotype SMG22.

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ABSTRACT

The common bean crop in dry areas is affected by the climate change and the low availability of nutrients that limits its yield. The objective of this review was to analyze the scope of the scientific literature on the use of inoculants based on Plant growth promoting rhizobacteria (PGPR) and humic substances (HS) in sustainable agronomic management of common bean crop and drought tolerance, for the generation of recommendations applicable to the production cultivation in dry areas from low tropic. An exploratory review on the use of PGPR and SH in common bean was carried out, since the first publications until December 2022 in the databases: Science direct, SciELO, SpringerLink, Scopus, Pubmed and Proquest. The co-inoculation of rhizobia with other PGPR was the most frequent technique in the reviewed articles. However, the combined application with SH allows greater tolerance to the water stress caused by drought. Rhizobia species most reported as efficient were *Rhizobium tropici*, *Rhizobium etli* and the strain CIAT 899 (*R. tropici*). In addition, the strain CIAT 899 was found to be the most useful in inoculant formulations for common beans under drought conditions in Brazil. In Colombia, only one registered product based on *Rhizobium phaseoli* was found for common bean, although there are no reports of evaluation of this strain under drought stress conditions.

Additional key words: sustainable agriculture; biological fertilization; humic substances; combined application; drought tolerance.



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RESUMEN

El cultivo de frijol común en zonas secas se ve afectado por el cambio climático y la baja disponibilidad de nutrientes que limita su rendimiento. El objetivo de esta revisión fue analizar el alcance de la literatura científica sobre el uso de inoculantes basados en rizobacterias promotoras de crecimiento vegetal (PGPR) y sustancias húmicas (SH) en el manejo agronómico sostenible del cultivo de frijol y la tolerancia a la sequía, para la generación de recomendaciones aplicables a la producción del cultivo en zonas secas del trópico bajo. Se realizó una revisión exploratoria sobre el uso de PGPR y SH en frijol, desde las primeras publicaciones hasta diciembre de 2022 en las bases de datos: Science direct, Scielo, Springerlink, Scopus, Pubmed y Proquest. La co-inoculación de rizobios con otras PGPR fue la técnica más frecuente en los artículos revisados. Sin embargo, la aplicación conjunta con SH permite mayor tolerancia al estrés hídrico ocasionado por la sequía. Las especies de rizobios más reportadas como eficientes fueron *Rhizobium tropici*, *Rhizobium etli* y la cepa CIAT 899 (*R. tropici*) es la más útil en formulaciones de inoculantes para frijol común en condiciones de sequía en Brasil. En Colombia, solo se encontró un producto registrado a base de *Rhizobium phaseoli* para frijol, aunque no existen reportes de evaluación de esta cepa bajo condiciones de estrés por sequía.

Palabras clave adicionales: agricultura sostenible; fertilización biológica; sustancias húmicas; aplicación conjunta; tolerancia a sequía.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most cultivated species in the world of the genus *Phaseolus* (Jiménez, 2019). The cultivation of this legume is of great interest for family farming and the peasant economy, due to its inclusion in the family basket and nutritional value, derived from its high content of protein, carbohydrates and some minerals (Abdulrahman *et al.* 2021). In addition, it is one of the most consumed foods in developing countries, which is why it is considered essential for global food security (Rezende *et al.*, 2018). This crop originally from America (De Ron *et al.*, 2019) was later introduced in Asian and African countries, adapting to new environmental and cultivation conditions. In 2020, Asia had the largest common bean cultivated area with 18.867.971 ha, followed by Africa (8.464.013 ha) and America (7.169.372 ha). However, the yield statistics place the European countries with the highest average, with (2.04 t ha⁻¹) followed by America (1.13 t ha⁻¹). In the case of Colombia, a cultivated area of 91.239 ha and a production of 113.875 t were obtained (FAOSTAT, 2021).

The *Phaseolus* genera, 50 species have been described, of which five have been domesticated: *P. lunatus*, *P. coccineus*, *P. vulgaris*, *P. polyanthus* and *P. acutifolius* that are part of the accessions conserved in the Genetic Resources Unit of International Center for Tropical

Agriculture (CIAT) (Debouck, 2021). In accordance with the above, currently the bean genetic improvement program of the CIAT has taken advantage of the genetic diversity of this heritage related to the common bean from double and triple interspecific crosses between *P. vulgaris*, *P. coccineus* and *P. acutifolius* to confer attributes of tolerance to high temperatures and drought, which in the future will allow expanding the life zones suitable for beans from the mountainous areas between 900 and 2,700 m a.s.l. (Debouck and Hidalgo, 1985), to the flat areas of the Colombian Caribbean (Burbano-Erazo *et al.*, 2021). The CIAT and Colombian Corporation for Agricultural Research (AGROSAVIA) alliance, advances in a project financed by KolFaci to obtain a variety of common beans that is tolerant to seasonal drought in warm zones, for which it is necessary to simultaneously generate technical recommendations for the sustainable management of the crop by small producers.

In this low tropical scenery, one of the main challenges common bean production faces is its vulnerability to climate change due to the impact of abiotic stress on yield, mainly prolonged droughts and high temperatures (Tofiño *et al.*, 2016; Perez *et al.*, 2019; Suarez *et al.*, 2020). It is estimated that drought is one of the factors that most limits crop production in the world

(Sooriyaarachchi *et al.* 2021), and in the case of beans it is decisive, since it has a negative effect on the morphophysiology of this plant (Chaves-Barrantes *et al.*, 2018). Added to this, the availability of nutrients under conditions of water deficit is deficient, microbial activity is limited and the participation of microorganisms as stimulating agents of the nutrient cycle in the soil decreases (Santillana, 2021).

Chemical fertilization is the most common in these areas. However, the sources and methods used are not adequate, since the plant assimilates less than 70% of the applied input and the rest is immobilized by the soil microbiota or is lost by volatilization and leaching, which is due to the way inefficient application or excess dose (Morales *et al.*, 2019). The wrong fertilization practices generate negative consequences on the environment, native microbial communities, and long-term soil fertility (Baweja *et al.*, 2020; Sellappan *et al.*, 2021).

In order to overcome these limitations, sustainable production strategies have been considered, which can mitigate the impact of abiotic stress and nutrient deficiency on common bean production. One of the strategies that stands out is the use of biological inoculants based on plant growth promoting rhizobacteria (PGPR), whose foundation lies in the ability of some soil bacteria to colonize plant roots and promote their growth, helping them in addition to tolerating biotic and abiotic stress through various mechanisms (Lakshmanan *et al.*, 2017). Some of these mechanisms correspond to the biological nitrogen fixation (BNF), through the formation of symbiotic structures such as nodules in the root of legumes to obtain available nitrogen (Colás-Sánchez *et al.*, 2018); the production of exopolysaccharides (EPS); the synthesis of phytohormones such as indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophores; and phosphate solubilization (Santillana, 2021).

Another sustainable production strategy corresponds to biostimulants such as humic substances (HS), which have been studied for their bioactivity in promoting plant growth and development. Within the effects generated by HS in plants, the induction in the proliferation of roots that modify the architecture of the root system is recorded (Chen and Avid, 1990; Façanha *et al.*, 2002; Zandonadi *et al.*, 2007; Dobbss *et al.*, 2010; Trevisan *et al.*, 2010; Canellas and Olivares, 2014; Canellas *et al.*, 2015) and tolerance to abiotic stress conditions (Guridi-Izquierdo *et al.*, 2017;

Veobides-Amador *et al.*, 2018; Kiran *et al.*, 2019). In addition, the H⁺-ATPase activity that facilitates the absorption of nutrients has been reported, as well as the like auxin effect, which stimulates plant growth (Nardi *et al.*, 2012; Canellas *et al.*, 2015; Nardi *et al.*, 2017). However, these effects are considered dependent on the source, dose, application method and plant genotype (Vaughan and Malcolm, 1985; Rodda *et al.*, 2006; Zandonati *et al.* 2007; Canellas *et al.*, 2015).

In this sense, both PGPR and HS constitute attractive and promising sustainable production strategies to contribute to the promotion of common bean growth and production under sustainable systems under drought conditions, since there are some studies that demonstrate their effectiveness as inoculants or biostimulants (Yanni *et al.*, 2016; Melo *et al.*, 2017; Figueiredo *et al.*, 2008a; Aserse *et al.*, 2020; Steiner *et al.*, 2020). Consequently, this systematic review aims to analyze the scope of the scientific literature on the use of inoculants based on plant growth promoting bacteria (PGPR) and humic substances (HS) in the sustainable agronomic management of bean cultivation and tolerance. To drought stress conditions, for the generation of recommendations applicable to crop production in the low tropics.

MATERIALS AND METHODS

An exploratory review of scientific literature on the use of plant growth promoting rhizobacteria (PGPR) and biostimulants based on humic substances (HS) in common beans was carried out using the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) methodology. This methodology consists of a review of the literature on the topic of interest with the purpose of drawing conclusions, considering the search engines and search terms, as well as inclusion and exclusion criteria. To search for articles that considered adverse conditions for the crop, such as drought or salinity stress in the science direct, SciElo, SpringerLink, Scopus, Pubmed and Proquest databases, from the first publications of each database until December 2022. The search was carried out taking into account the equation: (All (*Phaseolus vulgaris* L. and drought conditions, PGPR) or Research article) and the following inclusion criteria: a) That the genus and species of the plant be indicated "*Phaseolus vulgaris*" or common bean in title/abstract; b) original articles; c) plant growth promoting activity by rhizobia or rhizobacteria PGPR on *Phaseolus*

vulgaris L. reported in title/abstract, including under stress conditions; d) Bioactivity of humic substances informed in title/abstract; f) Studies published in Spanish, English or Portuguese. On the other hand, the following exclusion criteria were considered: A) Articles not available in full text. B) Incomplete information: articles that include *Phaseolus vulgaris* in the methodology, but do not describe the results obtained in this regard.

To extract the information, it was registered in a database created in Microsoft Excel® version 2013 and the title, author, country, year, journal of publication, humic substances used and their effect on the plant, rhizobia and rhizobacteria evaluated and their effect. On the plant as a result obtained were considered.

Analysis of data

After the implementation of inclusion and exclusion criteria for the search and selection of scientific research articles, a database was designed in the Microsoft Excel® v 2013 program, subsequently it was analyzed qualitatively and a synthesis of the documents was made.

RESULTS AND DISCUSSION

The exploratory search carried out until December 2022 allowed us to obtain 1139 records. From this information, 78 articles were obtained to review, of which 45 were included after reading them completely (Fig. 1).

According to the inclusion criteria, it was evidenced that the country with the highest production of scientific articles with useful information for this research was Brazil with 27% (12), followed by Cuba (4), Spain (4) and India (4) with 9% respectively; then Iran (3) and Egypt (3) with 7%, Belgium (2), Peru (2), Russia (2), China (2), Argentina (2) with 4% respectively and to a lesser extent Tunisia (1), Colombia (1) in 2019, Kenya (1), Mexico (1) and Ethiopia (1) with 2% respectively. This panorama shows that in Colombia only one study has been carried out with PGPR associated with *P. vulgaris* (Calero-Hurtado *et al.*, 2019).

Characteristics of the studies

Information related to PGPR + *P. vulgaris*, humic substances + *P. vulgaris* and PGPR + *P. vulgaris* +

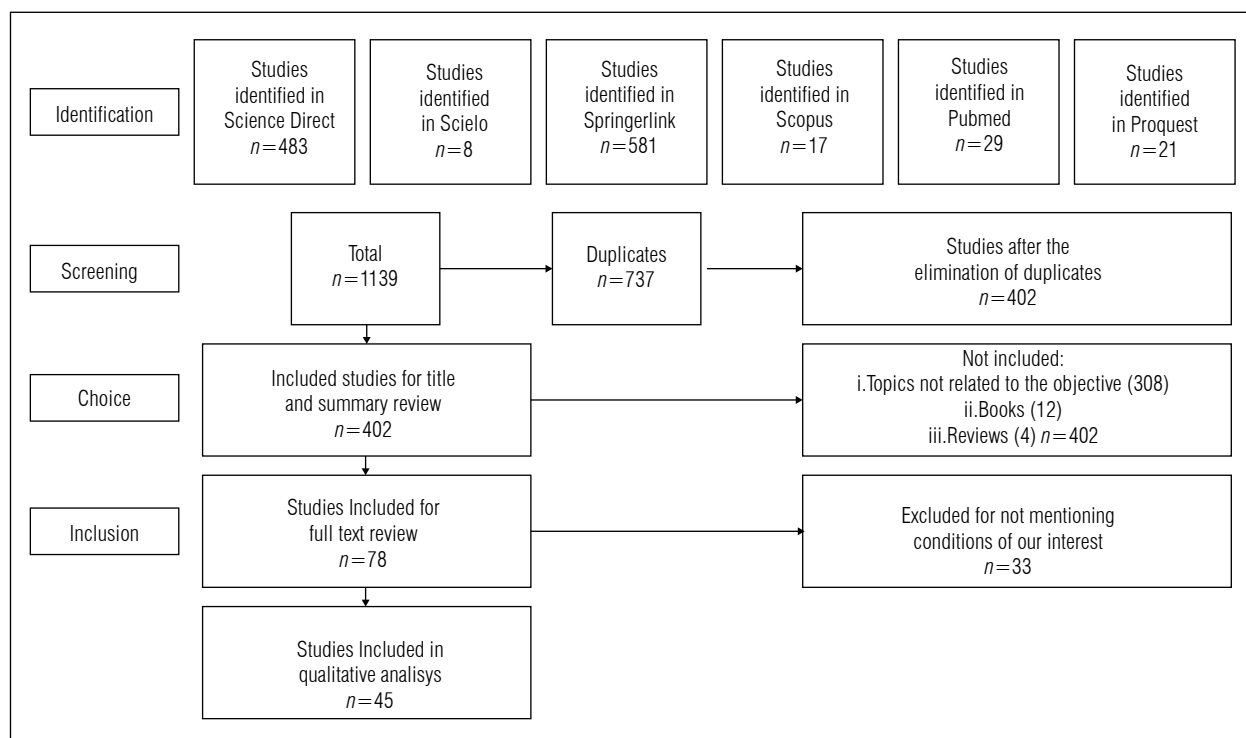


Figure 1. Diagram of the identification, selection and inclusion of studies from the exploratory search.

(dry stress or drug conditions or drought tolerance) was found since 1986 and a growth in interest in these topics is observed in the last six years (2017-2022) with a greater scientific production in the year 2022.

Of the articles included, which used the co-inoculation technique of rhizobia and other PGPR to evaluate their biofertilizing effect on the common bean crop, they corresponded to 51%. Likewise, it was found that 11% of the investigations evaluated the co-inoculation of fungi and other PGPRs other than

rhizobia, while the isolated application of rhizobia and PGPR was 13% and 18%, respectively.

The collected records indicate that the variables: increase in the amount of fixed nitrogen, number of nodules, dry weight of the shoot, nodules and root, in addition to the increase in tolerance to stress, were the most referred in relation to the promotion of plant growth of common bean plants due to the application of PGPR, inoculated or co-inoculated rhizobia, or the application of HS-based biostimulants individually or in conjunction with PGPR (Tab. 1).

Table 1. Effects of the techniques used for growth promotion and sustainable production of common bean plants.

Technique	Effect on plants of <i>Phaseolus vulgaris</i> L.	Percentage of articles that implemented the technique	Source
Co-inoculation	<ul style="list-style-type: none"> • Increase in the amount of fixed nitrogen, seed production, germination percentage, leghemoglobin concentrations, stimulation of nodulation, number of nodules, dry weight of shoot, dry weight of root and pods, number of pods, higher production of seeds per pod, efficiency in increasing the average number of leaves per plant, vegetative growth (stem diameter, dry weight of shoot, leaf area and protein content). • Increase in the quality of the harvested seeds in reference to their size, root branching, reduction in the incidence of important root and shoot diseases and acetylene reduction activities. • Allows a more prolonged and persistent exudation of flavonoids by the roots of the common bean. • Improve foliar membrane stability, minimizes water loss from common bean plants exposed to drought stress. • Improve nodulation and the relative index of chlorophyll under severe drought stress conditions. • Reduce the abortion rate of the pods in moderate drought conditions. • Improve drought tolerance of plants, growth and biomass and grain yield of beans. 	62%	Camacho <i>et al.</i> (2001); Remans <i>et al.</i> (2007); Remans <i>et al.</i> (2008); Figueiredo <i>et al.</i> (2008a); Figueiredo <i>et al.</i> (2008b); Dardanelli <i>et al.</i> (2008); Yadegari <i>et al.</i> (2008); Yadegari <i>et al.</i> (2010) Franzini <i>et al.</i> (2013); Hungria <i>et al.</i> (2013a); Diez-Mendez <i>et al.</i> (2015); Melo <i>et al.</i> (2017); Mercante <i>et al.</i> , 2017; Korir <i>et al.</i> (2017); Yadav <i>et al.</i> (2013); Colás <i>et al.</i> (2014); Vasconcelos <i>et al.</i> (2020); Kumar <i>et al.</i> (2016); Souza <i>et al.</i> (2017); Ferreira <i>et al.</i> (2018); Colás-Sánchez <i>et al.</i> (2018); Calero-Hurtado <i>et al.</i> (2019); Hidalgo <i>et al.</i> (2019); Negi <i>et al.</i> (2021); Steiner <i>et al.</i> (2020); Mortinho <i>et al.</i> (2022); Leite <i>et al.</i> (2022)
Isolated rhizobia inoculation	<ul style="list-style-type: none"> • Higher yields, nodules with higher nitrogenase activity and higher biomass. • Tolerance to drought stress indicated by the SWD (Surface Water Dynamic), increased seed yield 	13%	Suárez <i>et al.</i> (2020); Trabelsi <i>et al.</i> (2011); Cantaro-Segura <i>et al.</i> (2019); Yanni <i>et al.</i> (2016); Aserse <i>et al.</i> (2020); Alinia <i>et al.</i> (2022)
Inoculation of PGPRs other than rhizobia	<ul style="list-style-type: none"> • Significant increase in the length of the primary root, the number of secondary roots and the dry weight of the root. • Increase in the percentage of seed germination in adverse conditions. • Modifies the pattern of flavonoids exuded by the roots of the bean, increases the surface of the root and its dry weight. • - Increased absorption of magnesium, increased chlorophyll content and control of bacterial wilt 	18%	Dardanelli <i>et al.</i> (2012); Martins <i>et al.</i> (2015); Sabaté <i>et al.</i> (2017); Martins <i>et al.</i> (2018); Kumar <i>et al.</i> (2020); AlAli <i>et al.</i> (2021); Lastochkina <i>et al.</i> (2021); Velichko <i>et al.</i> (2022)
Application of humic substances	<ul style="list-style-type: none"> • Significant increase in root length, projective area and surface area, nodule number, nodule dry weight, N, P, Ca and Mg content, seed yield. • Significant improvement of the dry biomass of shoots and roots in plants, seed yield and its components 	7%	Qian <i>et al.</i> (2015); Ibrahim and Ramadan (2015); Machiani <i>et al.</i> (2019)

Meanwhile, 7% of the registered articles reported the use of biostimulants such as HS for bean cultivation, among which such as humic acids (HA) were found, from sources such as vermicompost, leonardite and organic matter, and some substances similar to HA from vermicompost (Tab. 1). From which the evaluation carried out by (Melo *et al.*, 2017) stands out, who carried out the joint application of the BR322, BR520, and BR534 strains of *Rhizobium tropici*, co-inoculated with the HRC54 strain of *Herbaspirillum seropedicae* and combined with HA obtained from vermicompost (Tab. 2).

In greater detail, it is evident that at an ecophysiological level, the use of PGPR and HS in the cultivation of common beans under drought conditions allow to counteract some effects caused by these conditions, such as changes in hormonal balance, increased of abscisic acid, decreased indole acetic acid, endogenous cytokinins, gibberellic acid and zeatin in leaf tissue. In this regard, the plant-microorganism interaction provides various beneficial effects on *P. vulgaris* L. under drought stress conditions as shown in table 2. However, in Colombia, there are no reports of the joint application of HS and rhizobia inoculated or co-inoculated with other PGPR in common beans, there is only one study carried out on guajiro bean plants (*Vigna unguiculata* L.) in which rhizobia co-inoculated with *Bacillus mycoides* were evaluated in joint application with humic acids extracted from charcoal. low lignite-type range (Valero *et al.* 2021).

Plant growth promoting activity of rhizobia and other rhizobacteria on *Phaseolus vulgaris* L.

According to the registered articles, information was found on the plant growth promoting activity of *P. vulgaris* L. from 16 rhizobia and 26 rhizobacteria (Tab. 3).

The most frequent species of rhizobia reported in the reviewed articles are *Rhizobium tropici* (29.5%), *Rhizobium* sp. (20.5%) and *Rhizobium etli* (15.9%). Some of the less frequent ones belong to the genera *Burkholderia* sp. and *Sinorhizobium* sp.

The table 3 shows that the species of other rhizobacteria, different of the rhizobia, with the highest number of records were: *Azospirillum brasilense* and *Bacillus* sp with a percentage of 12.7 and 10.9% respectively, followed by *B. subtilis* (9.1%), *Pseudomonas fluorescens* with (9.1%), *Pseudomonas* sp and *Bacillus amyloliquefaciens* with 7.3% and *Bacillus subtilis* strain different from the previous ones with 5.5%, while among the less frequent are *Paenibacillus polymyxa*, *Pseudomonas putida*, among others.

The identification of stage and cultivation conditions of scientific studies related to the use of PGPR and HS in common beans showed that 18% (8 articles) of the articles reviewed only reached the evaluation stage of the inoculation, co-inoculation of PGPR and/or use of HS under laboratory conditions, while 33% (15 articles) were evaluated both under greenhouse

Table 2. Rhizobia, others PGRP and HS used as biofertilizers and biostimulants for common beans under drought stress conditions.

Strains of <i>Rhizobia</i> and other PGRPs	Effect on <i>Phaseolus vulgaris</i> L. under drought conditions	Source
Cepas <i>Rhizobium tropici</i> BR322, BR520 and BR534 + <i>Herbaspirillum seropedicae</i> + HA	Increase in the dry mass of shoot and roots of the plants under water stress conditions compared to those not inoculated and fertilized with mineral N	Melo <i>et al.</i> (2017)
<i>Rhizobium radiobacter</i> , <i>Rhizobium etli</i> , <i>Rhizobium</i> sp.	Increased seed yield in the field under drought stress	Yanni <i>et al.</i> (2016)
<i>Rhizobium tropici</i> + <i>Paenibacillus polymyxa</i>	Increased growth, shoot dry matter accumulation, number of nodules and nodule dry matter	Figueiredo <i>et al.</i> (2008a)
<i>Rhizobium tropici</i> + <i>Azospirillum brasilense</i>	<ul style="list-style-type: none"> Improve the stability of the cell membrane of the leaf tissue and minimizes the loss of water from the leaf tissue of common bean plants exposed to drought stress. Improve nodulation and the relative index of chlorophyll under severe drought stress conditions 	Steiner <i>et al.</i> (2020)
<i>Rhizobium etli</i> HAMB13556 (HBR5), <i>Rhizobium Phaseoli</i> HAMB13562 (HBR10), <i>R. Phaseoli</i> HAMB13570 (HBR53) and rhizobia strains EAL428	<ul style="list-style-type: none"> Improve drought tolerance of plants, growth and biomass and grain yield of common beans 	Aserse <i>et al.</i> (2020)

Table 3. Rhizobia and others plant growth promoting rhizobacteria used in common beans (*P. vulgaris* L).

	Microorganism	Frecuency	Percentage
Rhizobia	<i>Rhizobium etli</i>	7	15.9%
	<i>Rhizobium tropici</i>	11	29.5%
	<i>Rhizobium cellulosilyticum</i>	1	2.3%
	<i>R. leguminosarum</i>	3	6.8%
	<i>Rhizobium pisi</i>	2	4.5%
	<i>Rhizobium</i> sp.	9	20.5%
	<i>Burkholderia</i> sp.	1	2.3%
	<i>Rhizobium gallicum</i>	1	2.3%
	<i>Sinorhizobium meliloti</i>	1	2.3%
	<i>Burkholderia fungorum</i>	1	2.3%
	<i>Rhizobium leguminosarum</i>	1	4.5%
	<i>Sinorhizobium</i> sp.	1	2.3%
	<i>Rhizobium radiobacter</i>	1	2.3%
	<i>Rhizobium Phaseoli</i>	1	2.3%
	<i>Bradyrhizobium elkanii</i>	1	2.3%
	<i>B. diazoefficiens</i>	1	2.3%
Rhizobacteria	<i>Azospirillum brasilense</i>	6	12.7%
	<i>Bacillus endophyticus</i>	1	1.8%
	<i>B. pumilus</i>	1	1.8%
	<i>B. subtilis</i>	4	9.1%
	<i>Paenibacillus lautus</i>	1	2.0%
	<i>P. macerans</i>	1	1.8%
	<i>P. polymyxa</i>	2	3.6%
	<i>Bacillus</i> sp.	6	10.9%
	<i>Bacillus subtilis</i>	3	5.5%
	<i>Pseudomonas putida</i>	1	1.8%
	<i>Pseudomonas fluorescens</i>	4	9.1%
	<i>Herbaspirillum seropedicae</i>	2	3.6%
	<i>Pseudomonas</i> sp.	4	7.3%
	<i>Xanthomonas</i> sp.	1	1.8%
	<i>Azospirillum lipoferum</i>	1	1.8%
	<i>Bacillus megaterium</i>	1	1.8%
	<i>Paenibacillus</i> sp.	1	1.8%
	<i>Pseudomonas entomophila</i>	1	1.8%
	<i>Bacillus amyloliquefaciens</i>	4	7.3%
	<i>Pseudomonas monteillii</i>	1	1.8%
	<i>Paenibacillus polymyxa</i>	1	1.8%
	<i>Chryseobacterium balustinum</i>	1	1.8%
	<i>Paenibacillus lentimorbus</i>	1	1.8%
<i>Herbaspirillum lusitanum</i>	1	1.8%	
<i>Pseudomonas syringae</i>	1	1.8%	
<i>Paenibacillus lentimorbus</i>	1	1.8%	

and field conditions. In addition, it was found that only 16% (7 articles) reported executing experiments under greenhouse conditions and then replicating them under field conditions.

Regarding the strains that have been used as inoculants to promote the growth of common beans, rhizobia were identified as individual active ingredients: *Rhizobium etli* and *Rhizobium* sp. (Suárez *et al.* 2008; Cantaro-Segura *et al.*, 2019), *Rhizobium leguminosarum* b.v. *phaseoli* (Mortinho *et al.*, 2022) and other PGPRs other than rhizobia: *Bacillus subtilis*, *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens*, *Herbaspirillum lusitanum* and *Pseudomonas syringae* (Sabaté *et al.*, 2017; Kumar *et al.*, 2020; Lastochkina *et al.*, 2021; Velichko *et al.*, 2022). Through the co-inoculation technique, the joint application of rhizobia and rhizobacteria were identified: *Rhizobium etli* + *Azospirillum brasilense*, *Rhizobium tropici* + *Bacillus endophyticus* (DSM 13796), *B. pumilus* (DSM 27), *B. subtilis* (DSM 704), *Paenibacillus lautus* (DSM 13411), *P. macerans* (DSM 24), *P. polymyxa* (DSM 36), *P. polymyxa* (Loutit L.) or *Bacillus* sp. (65E180), *Rhizobium tropici* + *Pseudomonas* sp., *Rhizobium tropici* + *Bacillus* sp., *Rhizobium* sp. + *Pseudomonas fluorescens*, *Rhizobium etli*, *Rhizobium tropici* + *Azospirillum brasilense* (Dardanelli *et al.*, 2008; Figueiredo *et al.*, 2008; Remans *et al.*, 2008; Yadav *et al.*, 2013; Ferreira *et al.*, 2018), *Rhizobium tropici* CIAT 899 + *Bacillus* sp., *Paenibacillus* sp., *Bacillus subtilis*, *Burkholderia fungorum*, *Pseudomonas* sp., *Pseudomonas entomophila*, *Bacillus amyloliquefaciens* (Vasconcelos *et al.*, 2020) *Rhizobium tropici* + *Azospirillum brasilense* (Souza *et al.*, 2017) *R. tropici* + *A. brasilense*. *R. tropici*

+ *B. subtilis*, *R. tropici* + *P. fluorescens*, *R. tropici* + *A. brasilense* + *B. subtilis*, *R. tropici* + *A. brasilense* + *P. fluorescens* (Mortinho *et al.*, 2022) *Rhizobium tropici* strain CIAT 899 (= SEMIA 4077) *Bradyrhizobium elkanii* y *B. diazoefficiens* (Leite *et al.*, 2022).

In Colombia, 12 bacterial inoculants formulated based on rhizobia were found (Tab. 4), taken from the table of inoculants registered with the Colombian Agricultural Institute (ICA) until November 2021, of which 8 are for soybean cultivation based on *Rhizobium japonicum*, *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* bv. *Phaseoli*, 3 for the cultivation of peas based on *Rhizobium phaseoli*, *Rhizobium leguminosarum* and *Bradyrhizobium japonicum*, and only one for the cultivation of common beans based on *Rhizobium phaseoli* (ICA, 2023).

Unlike Colombia, where there is a low supply of commercial inoculants for common beans, in Brazil, 51 registered inoculants based on rhizobia were found. All the strains used for the preparation of these inoculants correspond to the *Rhizobium tropici* species in different concentrations and formulations (Tab. 5). Among the *R. tropici* strains used for the formulation of these inoculants are: SEMIA 4077 (also known as CIAT 899) and SEMIA 4080, which are part of the SEMIA Collection (Agricultural Microbiology Section) of rhizobia of the Diagnostic Department and Agricultural Research (DDPA, acronym in Portuguese). Both strains are used in bean cultivation as nitrogen-fixing bacteria by symbiotic association and their behavior favors the assimilation of N by plants

Table 4. Rhizobia-based inoculants registered in Colombia.

Commercial product name	Active ingredient	Microbial concentration	Formulation type	Authorized crops
Ferbiol	<i>Rhizobium japonicum</i>	1·10 ⁹ CFU/g	Solid substrate	Soybean
Biagro	<i>Bradyrhizobium japonicum</i>	1·10 ⁴ CFU/mL	Liquid	Soybean
Bionitro	<i>Bradyrhizobium japonicum</i>	1·10 ⁹ CFU/g	Polvo	Soybean
Nitragin	<i>Rhizobium phaseoli</i>	4·10 CFU/g	Polvo	Pea
Nitragin	<i>Rhizobium phaseoli</i>	1·10 ⁸ CFU/g	Polvo	Common beans
Rhizobiol	<i>Rhizobium leguminosarum</i> bv. <i>Phaseoli</i>	1·10 ⁸ CFU/mL	Liquid	Soybean
Rhizobiol	<i>Rhizobium leguminosarum</i>	1·10 ⁸ CFU/g	Polvo	Pea
Biagro 10	<i>Bradyrhizobium japonicum</i>	2·10 ⁹ CFU/g	Polvo	Soybean
Rhizobiol	<i>Bradyrhizobium japonicum</i>	1·10 ⁸ CFU/mL	Liquid	Soybean
Rizoliq top	<i>Bradyrhizobium japonicum</i>	1·10 ⁹ CFU/mL	Seed treatment solution	Soybean
Ene-2	<i>Bradyrhizobium japonicum</i>	1·10 ⁹ CFU/mL	Concentrated suspension	Soybean
Fixor n	<i>Bradyrhizobium japonicum</i>	5·10 ⁷ CFU/mL	soluble concentrate	Pea

in soils with low fertility. These strains are released and authorized by the Ministry of Agriculture for industrial use and production by companies that produce inoculants (Embrapa, 2021a).

Table 5. Rhizobia-based inoculants for common beans registered in Brazil.

Active ingredient	Microbial concentration	Formulation type
<i>Rhizobium tropici</i>	1·10 ⁹ CFU/mL or g	Liquid and solid
<i>Rhizobium tropici</i>	1.5·10 ⁹ CFU/mL or g	Liquid and solid
<i>Rhizobium tropici</i>	2·10 ⁹ CFU/mL or g	Liquid and solid
<i>Rhizobium tropici</i>	3·10 ⁹ CFU/mL or g	Liquid and solid
<i>Rhizobium tropici</i>	1·10 ¹⁰ CFU/mL or g	Solid

In Brazil, some works have evaluated the SEMIA 4077 strain in co-inoculation with *Azospirillum brasiliense* and the SEMIA 4080 strain has been evaluated in co-inoculation with *Herbaspirillum seropedicae* and in joint application with HA under drought conditions, where the beneficial effects on bean plants, such as those described in table 1 (Melo *et al.* 2017; Steiner *et al.*, 2020).

Based on this review, it was possible to find information related to the use of plant growth promoters such as rhizobia and other plant growth promoting rhizobacteria (PGPR), as well as biostimulants such as humic substances (HS), its effects, the techniques of inoculation and co-inoculation of rhizobacteria and joint application of rhizobacteria and HS on *Phaseolus vulgaris* L. plants. It was possible to identify the countries with the highest production of related scientific material, the years with the highest production of information, the frequency of microorganisms used as biofertilizers, the strains used in drought conditions, culture conditions and microorganisms with projection for the biotechnological generation of new inoculants.

The growing production of related scientific material has been influenced by the need to implement sustainable alternatives that allow maintaining or increasing crop production and, in turn, reduce the damage caused by climate change to soil and agriculture. Consequently, it was found that Brazil is the country with the most information related to the use of PGPR for common bean culture. This is because it has public policies that contribute to sustainably improving agricultural production and minimizing the impact caused by climate change on crops;

considering soil organic carbon and biological nitrogen fixation as a research reference for more than 50 years (Embrapa, 2021a). This national commitment promoted by the state is reflected in its position as the first of 15 countries with the greatest potential to store carbon, invest in soil studies and guarantee sustainability. Additionally, Brazil has demonstrated its ability to produce common beans with an agro-ecological approach, that is, through the use of biological fertilization with products based on rhizobia bacteria, which are registered and released by legislation for industrial production, which is regulated by the Ministry of Agriculture, Livestock and Supply (MAPA) in the normative instruction SDA No. 13 of 2011 (Brazil, 2011).

In this context, this guideline has facilitated a high rate of grain exports and the recognition of Brazil as a major agricultural producer and world food exporter, producing nearly 239 million tons and exporting 123 million tons of grain in 2020, which represented a total of 37 billion dollars in 2020 and 419 billion between 2000 and 2020 (Aragao and Contini, 2021b). The foregoing suggests that, like Brazil, Colombia should take on new challenges in research, develop an optimal system that facilitates bioprospection and selection of promising microorganisms, the establishment of biofabric and the management of better technologies for the production of efficient inoculants for agriculture, since beans are a protein of great importance in areas vulnerable to climate change, since there is a population of producers of the peasant, family and community agriculture system, where the soils are poor in organic matter, without supplementary irrigation and with higher rates of food insecurity (Jiménez, 2019; Suárez *et al.*, 2020).

Among the alternatives implemented in recent years are the techniques of inoculation, co-inoculation of rhizobia and other PGPR, as well as the joint application of HS and rhizobia co-inoculated with other PGPR, with the purpose of producing common beans in a sustainable manner and counteract some effects caused by climate change such as drought that have allowed to maintain or increase the production of this crop, considering that beans are the most cultivated vegetable protein in areas of high vulnerability to variability and climate change, besides, its management current agronomy is inefficient, causing a great environmental impact, which decreases the availability and absorption of nutrients, which is critical in arid zones, limiting productivity (Hungria *et al.*, 2013a; Jiménez, 2019; Steiner *et al.*, 2020).

For the development of these techniques and culture conditions, the results of this review showed that research has initially focused on a laboratory evaluation phase of PGPR such as rhizobia with potential in biological nitrogen fixation (BNF) (Dardanelli *et al.*, 2008; Suárez *et al.*, 2008; Dardanelli *et al.*, 2012; Qian *et al.*, 2016; Kumar *et al.*, 2020; AlAli *et al.*, 2021; Lastochkina *et al.*, 2021) and a greenhouse phase (Remans *et al.*, 2007; Figueiredo *et al.*, 2008a; Figueiredo *et al.*, 2008b; Remans *et al.*, 2008; Franzini *et al.*, 2013; Diez-Mendez *et al.*, 2015; Martins *et al.*, 2015; Melo *et al.*, 2017; Sabaté *et al.*, 2017; Ferreira *et al.*, 2018; Hidalgo *et al.*, 2019; Aserse *et al.*, 2019; Vasconcelos *et al.*, 2020; Steiner *et al.*, 2020). Meanwhile, other research has focused on an agronomic evaluation of these effects in the greenhouse and subsequent field replication, where the effects of the use of rhizobia, their symbiotic relationship with common bean plants and the joint application of HS are evidenced, under controlled conditions (Remans *et al.*, 2008; Trabelsi *et al.*, 2011; Yadav *et al.*, 2013; Kumar *et al.*, 2016; Martins *et al.*, 2018; Aserse *et al.*, 2019). This indicates the need and importance in the Colombian dry Caribbean of exhausting the stages of bioprospecting, validation and agronomic response in the field of common bean plants by inoculating native isolates and PGPR strains with biotechnological potential, in addition to their integration with other biostimulants such as HS, which, based on decisive results in these phases in increasing production and crop yield, can consolidate a new biofertilizer product.

The co-inoculation technique stands out, which allows bean plants to assimilate a greater amount of nitrogen, due to the synergistic interaction of rhizobia and other PGPR such as *Azospirillum*, where there is greater growth, plant development and response to nodulation, so one of its most significant contributions is the yield in nitrogen fixation as demonstrated by Hungria *et al.* (2013a), who obtained a yield of 98 kg ha⁻¹, with an increase of 8.3% using *Rhizobium tropici* and 285 kg ha⁻¹ with an increase of 19.6% when implementing the co-inoculation technique with *Rhizobium tropici* and *A. brasilense* compared to the non-inoculated and nitrogen-fertilized treatment. Meanwhile, Leite *et al.* (2022) reported an increase in grain yield by 20%, in the co-inoculation treatment between the CPAC 7 strain of *Bradyrhizobium diazoefficiens* and the CIAT 899 strain of *Rhizobium tropici*. However, in the soils of the state of Cesar, located in the Colombian dry Caribbean, a production of 0.8 t ha⁻¹ of common beans is reported, obtained with the implementation of conventional agronomic

management (MinAgricultura, 2017), which leads to the development of research studies where the use of rhizobia inoculation techniques and co-inoculation with other PGPR are evaluated and consequently make a comparison with conventional management, in such a way that the application of this promising technique with local biological materials can be verified in the conditions of the Colombian dry Caribbean.

The information obtained from the reviewed articles and the tendency to search for organic products that can be used for agriculture, indicates the feasibility of advancing in the development of biofertilizers from the microbial strains with the greatest biotechnological potential for the promotion of plant growth of common bean and the identification of those most used in evaluations of drought stress conditions. In accordance with the above, the records issued by the Brazilian agricultural research company (EMBRAPA) coincide with those obtained from the review of scientific articles where the behavior of rhizobia in common beans under water stress conditions was evaluated, indicating that emerging microbial strains belong to the *Rhizobium tropici* species and that their behavior in bean crops under drought conditions provides benefits in terms of crop yield (Embrapa, 2021b). Additionally, *Rhizobium etli* stands out evaluated by Aserse *et al.* (2020) under drought conditions, which increased the growth and yield of the common bean crop.

In relation to the use of plant growth promotion techniques such as the inoculation and co-inoculation of rhizobia with other PGPR in joint application with HS for the production of common beans under drought conditions, the research carried out so far showed the benefits on the improvement of the yield of the seeds, the growth and development of the plant, the contribution in the decrease of the hydric stress and the reduction of the use of nitrogenous fertilizers (Yanni *et al.*, 2016; Melo *et al.*, 2017), thanks to biological nitrogen fixation mediated by efficient rhizobia strains that supply the nitrogen needs of this legume (Hungria *et al.*, 2013a), which contribute approximately 80% of fixed nitrogen in the world (Calero-Hurtado *et al.*, 2019). In this context, the rhizobia-legume interaction in drought conditions can be recognized as an alternative for recovery from soil deterioration because it allows the activation of the biological activity of the soil, maintaining its balance, improving the availability of nutrients and their assimilation by plants, which provides protection to

crops, favoring their growth and productivity under field conditions (Calero-Hurtado *et al.*, 2019; Aserse *et al.* 2020). In a study where strains of *Rhizobium etli* and *Rhizobium tropici* were used, subjecting common bean plants to water stress conditions, an improvement in biomass, quality in terms of number of nodules, plant size, seed, dry weight and yield of the bean crop was obtained, which was very similar to that treated with nitrogen fertilizers (Aserse *et al.*, 2020). This indicates that microorganisms can be implemented for the agronomic management of common bean crops under drought conditions in a sustainable manner and mitigate the use of chemical fertilizers, since the latter are expensive and several applications are required to achieve adequate yield, while that the use of microorganisms requires small quantities and lower costs, which generates a better cost/benefit ratio for the producer.

Additionally, it was observed that the co-inoculation of rhizobia with other rhizobacteria, such as *Rhizobium tropici* + *Azospirillum brasilense* under severe drought stress conditions, improve nodulation and the chlorophyll index (Steiner *et al.*, 2020), proving that the co-inoculation technique has greater beneficial effects on bean plants, such as increased germination rate, better establishment of rhizobia, hormonal regulation. The foregoing could serve as a basis for the development of future research in Colombia where the use of this technique is implemented in bean plants in the edaphological and drought conditions of the Colombian Caribbean, since this technique apart from being one of the emerging technologies that contributes to sustainable agriculture, could be integrated into the agroecological management of the crop, due to the fact that the majority of bio-inputs in Colombia are classified as biological control agents, that is, that the bio-inputs registered with the ICA counteract the diversity of pests on several species of plants, but they do not function as growth promoters (ICA, 2023).

In Colombia, inoculants based on rhizobia have been registered for legumes, among which are soybeans, peas and common beans, highlighting soybeans as the crop to which most inoculants records have been attributed, while for common beans only It has a registered inoculant (Nitragin) based on *Rhizobium phaseoli*, of which there is no report of its use in drought conditions. However, HAMBI3562 and HAMBI3570 strains of *Rhizobium phaseoli* have been reported as effective in promoting common bean growth under water stress conditions. In addition, they have been

evaluated in co-inoculation with other rhizobia, obtaining beneficial effects that mitigate the use of N-based fertilizers (Aserse *et al.*, 2020); indicating that a single product for the country does not respond to the diversity of the bioclimatic supply of the different bean life zones, such as the Colombian dry Caribbean, which reflects the need to generate research with the aim of creating inoculants with greater specificity environment based on rhizobia for the improvement of the productive conditions of this legume (ICA, 2023). Additionally, the existing inoculants in Colombia are mostly powder and liquid, which have their advantages and disadvantages; the inoculation of products in solid formulations are based on peat or other soil conditioners that could stimulate plant growth, increase microbiological activity, aeration and soil moisture retention, however, its inoculation in seeds requires a solution sugary or sticky; while the inoculation of liquid products, unlike powdered products, does not require the use of a sugar or adhesive solution, since most contain substances that facilitate their adhesion to the seed (Hungria *et al.*, 2013b). In this sense, in the areas with the greatest lag in development where the cold chains are not maintained and both the irradiation and the density of UV rays is high, it is necessary to develop studies of mixed formulations that allow counteracting drought conditions. This panorama contrasts with the data of the productive system in Brazil, where bean cultivation is recognized as one of the most relevant for agriculture and has 51 records of bioproducts based on rhizobia, with solid and liquid formulations that allow a shelf life. longer, more effective in the field and its use depends on the forms of inoculation in the seed or in the furrow (Embrapa, 2021).

Despite the benefits of the symbiotic association of rhizobia with common bean plants, this rhizobia-legume interaction can be affected by the content of organic matter (OM) in the soil, one of the main indicators of soil quality for beans and that is related to the water retention capacity of the soil; under drought, low water availability in the profile decreases microbial activity and nutrient retention compared to humid conditions (Franco, 2017). The OM content varies according to the type of soil, in sandy and desert soils it is less than 1%, in the first 15 cm of mineral agricultural soils it is 4% and it increases to more than 50% in organic soils (Ayala *et al.*, 2018). In soils of the State of Cesar, located in the Colombian dry Caribbean, an OM content of 0.97% was reported, which increased to 1.40% due to the use of efficient practices after three years of treatment,

demonstrating that it is a limitation that can be improved through crop management techniques such as the incorporation of OM (Murillo *et al.*, 2014). Therefore, it is recommended that, for the conversion process from conventional to sustainable agriculture, that is, where rhizobia-based inoculants are used, prior soil analyzes are carried out to determine the OM content, a previous management practice that allows to improve the soil cover and thus obtain an increase in OM, in such a way that the drought is less drastic and consequently contributes to the survival of the inoculated PGPR.

Regarding the rhizobia used in common beans, it is evident that they present specificity for the genotype of the plant, which implies that, although a soil is diverse in rhizobia species, it does not mean that symbiotic associations with the plants will be established, limiting the formation of nodules, which has repercussions on family and community peasant agriculture of local varieties that present greater genetic variability (Shamseldin and Velásquez, 2020). In addition, for the varieties of common bean that have been adapted to the conditions of the Colombian dry Caribbean, there are no studies on efficient strains in the BNF. In this sense, to implement and formulate inoculants based on rhizobia and other efficient rhizobacteria in promoting growth for common bean varieties established under drought conditions in the dry Colombian Caribbean, as well as for the adaptation of bean genotypes with characteristics commercial interests, bioprospection is necessary that involves the development of selection experiments of new isolates of rhizobia adapted to the edaphological and edaphoclimatic conditions, as well as the use of biological resources conserved in the nation's germplasm banks guarded by national entities such as AGROSAVIA that have been obtained from warm areas, that are efficient in terms of BNF in drought conditions in both laboratory, greenhouse and field conditions; Just as in Brazil, the CIAT 899 strain of *Rhizobium tropici* is recognized for its ability to tolerate environmental stress conditions such as drought and has been authorized as an inoculant for its usefulness in bean cultivation (Brazil, 2011; Gomes *et al.*, 2019).

Within the consolidated lines of research are on the one hand in agricultural microbiology the use of PGPR to promote plant growth and on the other agricultural chemistry in the use of HS extracted from different sources. However, there is a growing interest in the development of emerging research that

integrates sustainable production techniques that are already consolidated, such as the use of rhizobia-based biofertilizers, other PGPRs, and biostimulants such as HS, with the purpose of maintaining or increasing the production of crops sustainably. Therefore, for the cultivation of common beans under drought conditions in the Colombian dry tropics, the evaluation of rhizobia strains with emerging techniques such as co-inoculation with other PGPRs and their joint application with HS is recommended, in such a way that the results obtained serve as scientific support for biofactories, which allow the design and formulation of new efficient mixed inoculants based on PGPR and HS. This calls for the prioritization of university-company-state alliances and the participation of producers in the stages of the development route: proof of concept, development and registration of a comprehensive inoculant for common bean production and reducing the gap of technological lag that Colombia's dry Caribbean suffers.

CONCLUSION

According to the results obtained in this review, it is concluded that: i) the use of inoculated and co-inoculated PGPR in joint application with biostimulants such as HS is a viable alternative in the generation of new bioproducts that minimize the impact caused by climate change, agricultural and livestock activities and some of their effects such as the drought that characterize the State of Cesar and in general, the dry Colombian Caribbean; ii) rhizobia species such as *Rhizobium tropici* and *Rhizobium elii* are the most effective and have demonstrated their ability to improve the yield and development of the bean crop, the former being the most recommended for the sustainable management of common beans in drought conditions; iii) studies in Colombia on the use of rhizobia and other PGPR both inoculated, co-inoculated and in joint application with HS are scarce, which makes evident the need to develop basic and applied research in the search and selection of rhizobia isolates, as well as other native PGPRs, the evaluation of rhizobia strains and other PGPRs from the biological resource banks of Colombia in the laboratory, the evaluation of inoculation and co-inoculation techniques of rhizobia and other PGPRs such as *Azospirillum brasilense*, as well as the joint application of PGPR with biostimulants such as HS in greenhouse and field conditions in drought conditions with a view to the development of mixed inoculants for dry areas such as the Colombian dry Caribbean, which contribute to crop

yield, counteract adverse drought conditions, favor water economy of the crop, minimizing the use of nitrogenous fertilizers and the promotion of adequate sustainable agronomic management which allows contributions to food security while preserving the carbon footprint provided by *P. vulgaris* L.

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