BIOSCIENCE JOURNAL

# RHIZOBIA INOCULATION INCREASES PEA GRAIN YIELD: AN OVERVIEW AND CHALLENGES

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**How to cite:** VALENCIA, H. A. M., et al. Rhizobia inoculation increases pea grain yield: an overview and challenges. *Bioscience Journal*. 2024, **40**, e40034. https://doi.org/10.14393/BJ-v40n0a2024-67453

**Abstract**
The aim of this study was to evaluate the contribution of inoculation with microorganisms, mainly rhizobia, on pea (Pisum sativum) production using a systematic literature review approach and a comparative analysis of grain yield to provide information to fill gaps in nontraditional regions of cultivation for this legume. A systematic search strategy was implemented, targeting papers published in scientific journals accessible through the Web of Science® (WoS) database spanning from January 1990 to April 2022. The search terms "Pisum sativum," "inoculation," and "strain" were used. The differences between the pea grain yields from plants inoculated with nitrogen-fixing microorganisms and those from noninoculated plants (control) were analyzed using thirteen field-scale studies. Overall, pea inoculation/coinoculation promoted a greater grain yield (3118 ±210 kg ha$^{-1}$) than noninoculation (2338± 127 kg ha$^{-1}$), showing the importance of biological nitrogen fixation for legume crop production. In the pursuit of reducing pea production costs, emphasis has been placed on inoculation, highlighting the importance of comprehending the symbiotic relationship between peas and Rhizobium. Furthermore, this research aimed to explore the interaction between Rhizobium and selected microorganisms known to enhance plant growth to identify optimal combinations to boost pea productivity.

**Keywords:** Bibliometric analysis. Biological nitrogen fixation. *Pisum sativum*. Rhizobacteria. *Rhizobium*.

## 1. Introduction

The number of grain-producing pulses belonging to the Fabaceae family, which are rich in proteins, fibers, minerals, and vitamins, is increasing because, today, the production of vegetables and animal sources is essential for reducing world hunger because it is a more affordable and simpler method for accessing vulnerable individuals. Among crops, the pea (*Pisum sativum* L.) stands out due to its versatile use as a sustainable vegetable because both its fresh and dry seeds serve as a protein-rich food for humans and animals as pulses and feed, respectively.

Pea is one of the oldest crops in the world and originated in the Middle East with an ancient history of domestication; it is the third most cultivated food legume in many temperate regions around the globe (Choudhury et al. 2007; Choudhury et al. 2011; Ferhi et al. 2017; Bourion et al. 2018). The total pea production area increased from 6.0 million ha in 2000 to 7.2 million ha in 2019. Based on official, semiofficial, estimated or calculated data, this may be due to the growing trend of vegetarian diets and industrial demand. In contrast, in Brazil, the area cultivated with peas decreased from 14767 ha in 1988 to 711 ha in 2019 (FAO 2021).

Peas are symbiotic partners of nitrogen-fixing diazotrophic bacteria, e.g., *Rhizobium leguminosarum*, *R. anhuiense*, *R. indicum* and *R. hidalgonense* (Fesenko et al. 1995; Rahi et al. 2020; Suproniene et al. 2021), and due to this partnership, they are self-sufficient in terms of nitrogen for growth and grain production. Annually, of the total nitrogen (N) input in cultivable land, approximately 25% is represented by biological nitrogen fixation (BNF), which is obtained through the symbiosis of pulse and oilseed legume (Fabaceae) crops and prokaryote fixer microorganisms (Herridge et al. 2008). Even though nodulation has a high metabolic cost for *Rhizobium* and the legume, better symbiosis could be one strategy to boost this crop (Voisin et al. 2007).

In Brazil, microbial inoculants containing rhizobia have been on the market for more than 50 years (Bomfim et al. 2021). Hence, the inoculation of pea seeds with *Rhizobium* inoculants is an option for decreasing the use of nitrogen fertilizers since they are among the leading causes of agriculture-related greenhouse gas emissions and energy consumption reduction (Egamberdiyeva 2008; Galloway et al. 2008). According to data from different geographical regions of the world, the use of pea BNF in the range of 47–130 kg N $ha^{-1}$ $year^{-1}$ (Peoles et al. 2009). Rhizobia inoculation of legumes can be an economic practice, for instance, a study carried out under conditions in the South Region of Brazil showed a positive effect of pea inoculation with different rhizobial strains, which produced grain amounts similar to those resulting from the addition of 200 kg N $ha^{-1}$ (Muniz et al. 2017). However, the reaction between plants and microbial inoculants depends on various factors, such as the presence of native rhizobia populations due to strain competitivity (Fesenko et al. 1995), N availability, physical and chemical limitations of the soil and climatic conditions (Uyañoz and Karaca 2011). The complexity of factors influencing the population size and symbiotic performance of pea rhizobia in soils was highlighted in a study using 114 soils from Mediterranean-type southern Australian environments (Drew et al. 2012) and contrasting Tunisian soils (Hachana et al. 2021).

The interaction between the host and bacteria is not always efficient, probably due to the evolving symbiotic process. By some means, there are specific dynamics in terms of fixation efficiency by the plant that could change its interaction with the inefficient strains (Oono et al. 2011). The differences are even more specific when wild peas are compared with domesticated peas, and wild peas exhibit greater interactions with symbionts (Mutch and Young 2004). Thus, strains should be selected using various host plant types, regardless of which plant is evaluated. For instance, lentil *(Lens culinaris)* inoculation with selected rhizobial strains improved nodulation and shoot N uptake, increasing grain production compared to that in noninoculated plants (Tena et al. 2016). A study in which peanut seeds were not inoculated with rhizobia revealed the effects of the genotype and dose of biofertilizers on the number of nodules (Julião et al. 2022).

In our study, pea was chosen because it is considered an important multifunctional legume that can be consumed as fresh pods and dry and green grains as a source of protein. In addition, this crop has been studied for a long time for its ability to increase grain production due to rhizobium inoculation. The key hypothesis of our study was that microbial inoculation/coinoculation contributes to pea production; thus, our goals were to perform a systematic review of the literature and a comparative analysis of grain yield, aiming to provide information to fill gaps that still exist in nontraditional regions of cultivation of this legume.

## 2. Material and Methods

### Systematic review

The main steps of our study were based on a bibliographic search using the Problem/hypothesis, Intervention, Comparison, and Outcome (PICO) guidelines. For the problem/hypothesis formulation, we investigated whether N from BNF is sufficient to increase grain yield compared to that from noninoculated *Pisum sativum* crops.

According to Toronto and Remington (2020), the methodology comprises the following five steps: i) problem formulation to clarify the study state; ii) literature search, using a comprehensive and replicable search strategy to collect data; iii) a data evaluation stage, in which the methodological quality and relevance of selected literature are appraised; iv) a data analysis stage, which includes data abstraction, comparison, and synthesis; and v) a presentation stage, in which the interpretation of findings and implications for research are developed.

For primary report selection, the review's search strategies, which obeyed the guidelines emphasized by Liberati et al. (2009). Data were collected from papers published in scientific journals in English through a bibliographic review using the Web of Science® (WoS) database. The following search terms were used: (*Pisum sativum*) AND (inoculation) AND (strain). The citations of articles published in the WoS database from January 1990 to April 2022 were used to summarize the initial data and are displayed graphically.

**Analysis of studies**

A total of 177 papers were retrieved from the WoS database. The following variables were used for data evaluation and selection based on the inclusion criteria: grain yield (kg $ha^{-1}$), total plant height (cm), fresh weight (g), $N_2$ fixation (mg $plant^{-1}$), dry weight (g), shoot dry biomass (g $plant^{-1}$), photosynthesis (mg $CO_2$ $m^2$ $s^{-1}$), stem height (cm), leaf number, pod length (mm), number of pods per plant, chlorophyll-a, chlorophyll-b, total chlorophyll, number of seeds per pod, fresh weight of seeds (g), dry weight of seeds (g), root length (cm), number of nodules, lateral roots (cm), fresh nodule weight (g), dry nodule weight (g $plant^{-1}$) and root dry weight (g $plant^{-1}$).

For the comparative analysis, the search was expanded to include Google Scholar, Springer-link and other publishers. The criteria established for selecting articles in this search were pea field-scale studies involving inoculation with nitrogen-fixing bacteria (*Rhizobium* AND/OR *Azospirillum*) and control treatments with grain yield data, which were standardized to kg $ha^{-1}$. A few additional papers published outside the WoS database between January 1990 and April 2022 were also included.

**Data analysis and presentation**

To analyze the textual corpus, including the title, abstract and keywords of the papers, Iramuteq software (designed for multidimensional text and questionnaire analysis) (Ratinaud 2008) was used. Data on pea grain yield (averages) from the papers identified in the literature search were converted to kg $ha^{-1}$. Descriptive statistical analysis was conducted, and a confidence interval (CI, 95%) was applied for the sample mean ($\alpha$=0.05). To interpret the findings from articles published between January 1990 and April 2022 in the WoS database, mapping analyses were conducted. This involved generating network, density and overlay visualization maps. Factor correspondence analysis (FCA) was conducted by employing the Reinert textual clustering method, which enabled the generation of a word cloud graph, illustrating the frequencies of search terms based on their size and density (Ratinaud 2008).

## 3. Results

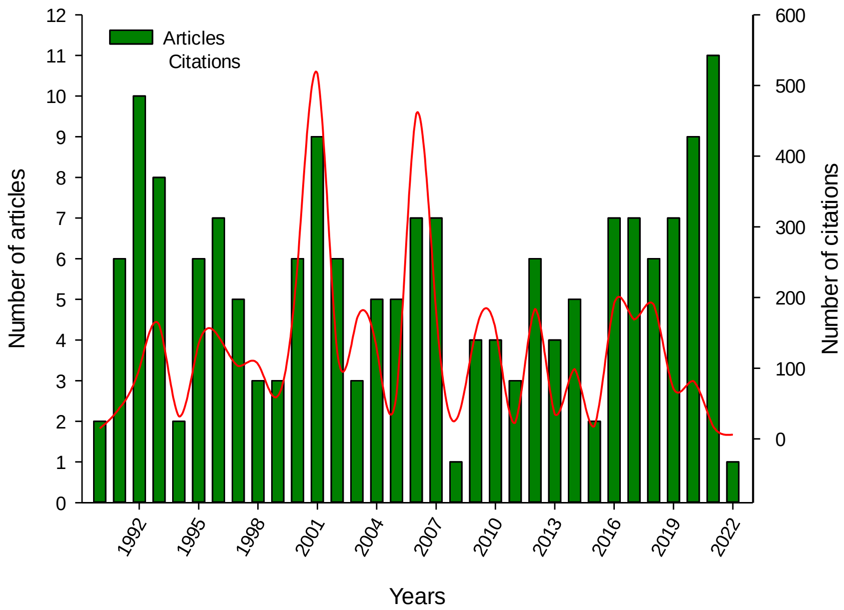
**Search results**

A total of 177 articles garnered 4,219 citations collectively in the WoS database, averaging 23.7 citations per paper. Notably, in 2001, nine publications with 517 citations were identified (Figure 1a).

Regarding the countries' contributions to studies related to pea symbiosis and their partnerships and collaboration links, India had the greatest number of papers, seven (7); followed by the USA, with six (6) papers; Canada and Pakistan, with four (4) papers each; Russia and Denmark, with three (3) papers each; and the People's Republic of China, Sweden, the Czech Republic, Egypt, Australia and Poland, with two (2) papers each (Figure 1b).

In the search for pea inoculated with microorganisms, when applying the criterion to filter publications to include only studies with one or more quantitative variables, twenty-six articles authored by researchers from sixteen countries were identified (Table 1).

**A**




**B**

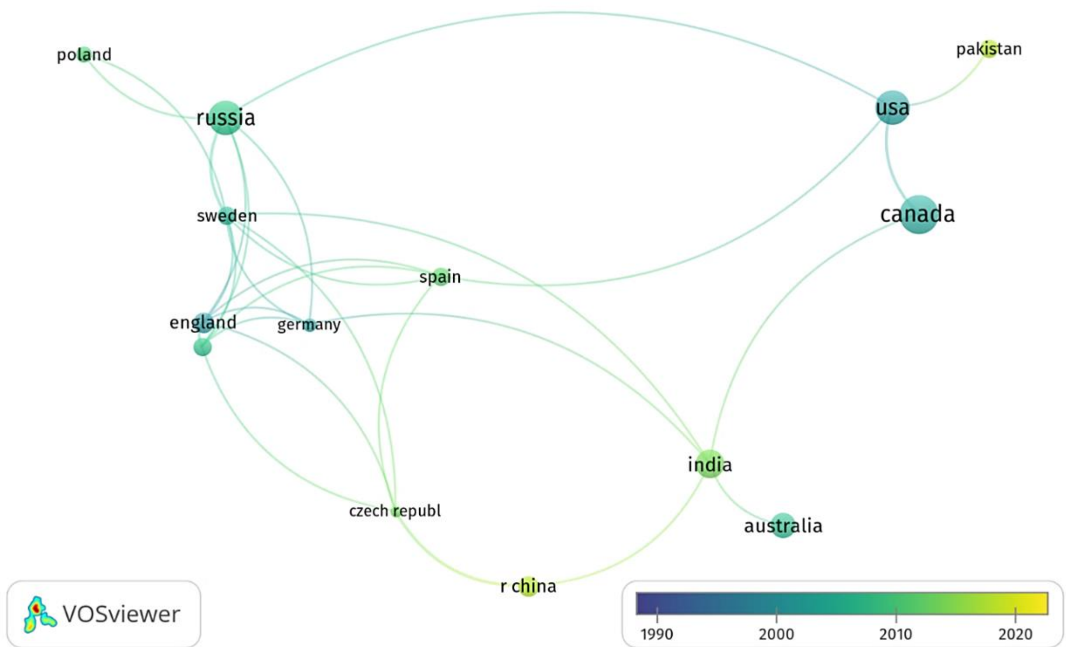



**Figure 1.** Results from the search of the papers by ("*Pisum sativum*") AND (inoculation) AND (strain) used in Web of Science® into WoS database from 1990 to April 29, 2022 (n=177). A - Number of articles vs number of citations per year and B - density visualization of the countries that contributed with the number of papers. The larger the size of the bubbles, the greater the contribution.

The outstanding surge in studies on pea inoculation with microorganisms published by researchers from India may indicate a response to the dietary preferences of the vegetarian population and the exploration of economically viable sources of grain protein. In Canada, in addition to the demand for human food, the availability of commercial inoculants in the country and favorable climate for the pea plant may explain why more field studies are showing the pea response to inoculation with *R. leguminosarum* (Table 2).

**Table 1.** Articles of pea (*Pisum sativum*) after the first selection. Search from 1990 to April, 2022.

| | Country | Treatment | Main Results | References |
|---|---|---|---|---|
| 1 | India | *Bacillus subtilis* RhStr _71 | Increases fresh weight, dry weight, length under salinity stress. | Gupta et al. (2021) |
| 2 | Pakistan | *P. fluorescence* | Increase shoots and root lengths, fresh biomass of shoots and roots under lead toxicity. | Shabaan et al. (2021) |
| 3 | Pakistan | *R. pisi* + ácido salicílico + abscisico | Attenuate drought-induced inhibition of plant growth and biomass. | Bashir and Naz (2020) |
| 4 | Russian Federation | *R. leguminosarum* bv viciae | Improvement of biomass, nodulation, $N_2$ fixation, uptake of N and nutrients under water deficit or Cd toxicity. | Belimov et al. (2020) |
| 5 | India | *Bacillus thuringiensis* + *Azotobacter chroococcum* | Higher percentage of germination. | Singh et al. (2019) |
| 6 | India | *Scytonema bohneri* + *Nostoc commune* + *Aphanothece stagnina* | Greater root response and root length. Longer seedling length. | Gayathri et al. (2017 |
| 7 | Peru | *R. leguminusarum* | Higher nodulation efficiency, nodulation speed. | Moreno-Chirinos et al. (2016) |
| 8 | Saudi Arabia/ Egypt | *Enterobacter cloacae+ R. leguminosarum* | Higher length and dry weight. | Khalifa et al. (2016) |
| 9 | Saudi Arabia | *B. megaterium* BMN1 | Longer primary root length, number of secondary roots and dry root weight. | Khalifa and Almalki (2015) |
| 10 | India | *P. putida* + *R. leguminosarum* | Higher growth, shoot nutrients and quantity and quality of seeds. | Ahmad et al. (2013) |
| 11 | India | *Pseudomonas sp* + *R. leguminosarum* | Better plant growth, iron acquisition and nutrient absorption. | Mishra et al. (2012) |
| 12 | China | *R. leguminosarum* | Higher fixed nitrogen and seed yield. | He et al. (2011) |
| 13 | India | *R. leguminosarum* + *B. thuringeinsis* | Better plant growth and nodulation. | Mishra et al. (2009) |
| 14 | Tunisia | *R. leguminosarum* | Biological control of *Orobanche crenata*. | Mabrouk et al. (2007) |
| 15 | Canada | *R. leguminosarum* | Greater nodulation and nodular mass. | Chemining'wa and Vessey (2006) |
| 16 | Bulgaria | *R. leguminosarum* + *Glomus mosseae* or *G. intraradices* | Increase growth, photosynthetic rate, nodulation, nitrogen fixing activity at low phosphorus. | Geneva et al. (2006) |
| 17 | Canada/ Brazil | *R. leguminosarum* | Rapid nodulation at 20 ºC. | Lira Junior et al. (2005) |
| 18 | Australia | *R. leguminosarum* | Shoot weight higher. | Ballard et al. (2004) |
| 19 | Canada | *Pseudomonas fluorescens, Serratia* spp. *and Bacillus* spp. | Reduce diseases by Pythium or Ascochyta, by Rhizoctonia and Fusarium. Increased seedling survival and shoot dry weight. | Wang et al. (2003) |
| 20 | Sweden | *R. leguminosarum* + *P. fluorescent* | Higher stem height, root length and dry weight. | Kumar et al. (2001) |
| 21 | Canada | *Bacillus subtilis and B. polymyxa* | Reduce the rot of *Pythium* in seeds and roots. | Hwang et al. (1996) |
| 22 | Germany | *Pantoea agglomerans* | Stimulates pea root growth and protects young plant roots against fungal pathogens. | Höflich and Ruppel (1994) |
| 23 | USA | *Pseudomonas cepacia* | Biocontrol of Pythium damping-off and root rot of *Aphanomyces*, higher yield. | King and Parke (1993) |
| 24 | Canada | *R. leguminosarum* + *P. syringae* + *P. putida* | Increase shoot, root and total plant weight. | de Freitas et al. (1993) |
| 25 | Czech Republic | *R. leguminosarum* | Increase dry matter of the shoot. | Šimon (1991) |
| 26 | India | *R. leguminosarum* + | Potted soil. Higher grain yield. | Jauhri et al. (1979) |

*Azotobacter chroococcum*

Using VOSviewer software, the twelve most frequent words with high density or frequency were the following: “*Pisum sativum*”, “Pea”, “Inoculation”, “Nitrogen Fixation”, “Nodulation”, “*Rhizobium”,* “Growth”, “PGPR”, “Rhizosphere”, “Strains”, “Plant-growth*”* and “Rhizobacteria", which were identified via the updated search (Figure 2a).

While searching using Iramuteq software and the strategy defined by the keywords, the timeline shows that the frequency of the searched terms occurs in the following order, as shown in parentheses (n): first, the foremost terms are: "pea" (167) and "plant" (125), second, "strain" (88), "growth" (87), "soil" (75), "inoculant" (73), third, "*Rhizobium*" (68), "nodulation" (51), "root" (49), "field" (47), "seed" (45), "yield" (40), "nodule" (40) and "increase" (38), fourth, "*leguminosarum*" (38), "bacterium" (37, "promote" (32), "nitrogen" (32), "rhizobia" (27), "fixation" (25) and "inoculate" (22). Among the less ones, there are the following stands out: "co-inoculation" (15), "drought" (14), "cyanobacteria" (6) and "nitrogenase" (5) (Figure 2b).

From the plotted similarity analysis of textual keywords used in the search from 1990 to 2022 in the database, a graphical representation was derived. The results of the similarity analysis revealed that two primary nodes were closely intertwined. The primary key term representing "pea" predominantly stems from the following branches: "*Rhizobium*" and "Fixation," "Bacterium," "Seed," "Soil," "Strain," "Nodulation," and "Field". The second node, represented by the term "Plant", is associated with the following two thickest branches: "Growth", which is simultaneously linked to the "Field" and "*Leguminosarum*" branches (Figure 3).

**Table 2.** Description of the field-scale studies and grain yield (kg ha$^{-1}$) of pea (*Pisum sativum*) included in the comparative analysis of non-inoculation (control) versus inoculation with nitrogen-fixing bacteria.

| | Country | Treatments inoculated (Nº) | Control | Inoculated | Reference |
|---|---|---|---|---|---|
| | | | _Grain yield (kg ha$^{-1}$)_ | | |
| 1 | Turkey | *Rhizobium leguminosarum* (4) | [a]2040 | 3580-5740 | Almaca et al. (2021) |
| 2 | Pakistan | *Azospirillum+Agrobacterium tumefaciens* (1) | 758 | 1443 | Ejaz et al. (2020) |
| 3 | Egypt | *Rhizobium* sp. (1) | 2205 | 2854 | Arafa et al. (2018) |
| 4 | Croatia | *R. leguminosarum* bv. Viciae (2) | [b]1702 | 1840- 2098 | Rapčan et al. (2017) |
| 5 | Brazil | *R. leguminosarum* bv. Viciae (8) | 762 | 1006-1170 | Muniz et al. (2017) |
| 6 | USA | *Rhizobium* sp. (3) | 1352-1492 | 1445-1567 | Huang et al. (2017) |
| 7 | Croatia | *R. leguminosarum* bv. viciae (2) | 2768-3129 | 3098-3607 | Uher et al. (2010) |
| 8 | Bangladesh | *Rhizobium* sp. (6) | 1200 | 1530-1900 | Ahmed et al. (2007) |
| 9 | Bangladesh | *Rhizobium* sp. (9) | 1350 | 2530-3160 | Rabbani et al. (2005) |
| 10 | Australia | *R. leguminosarum* bv viciae (2) | 780-2150 | 920-2870 | SLATTERY et al. (2004) |
| 11 | Canada | *R. leguminosarum* bv viciae (18) | 2200-3850 | 2610-5800 | Clayton et al. (2004) |
| 12 | Canada | *R. leguminosarum* bv. *Viciae* (26) | 1040-5110 | 1320-5320 | Kutcher et al. (2002) |
| 13 | Canada | *R. leguminosarum* bv. *Viceae* (3) | 2650-2890 | 2690-2960 | Begum et al. (2001) |

Number of treatment inoculated are given in parenthesis. [a]Data were converted from g plant$^{-1}$ to kg ha$^{-1}$; [b]data were converted from g m$^{-2}$ to kg ha$^{-1}$.

## Analysis comparative of the grain yield

The grain yield averages reported in the studies were converted to kg ha$^{-1}$, and the descriptive statistical parameters were calculated for two groups: noninoculated (control) and inoculated with microorganisms. Overall, inoculation/coinoculation of pea promoted a greater grain yield (2887 ±139 kg ha$^{-1}$) than noninoculation (2240± 120 kg ha$^{-1}$), showing the importance of biological nitrogen fixation for legume crop production. According to data from field studies (n=85) from 1990 to April 2022, pea inoculation with *Rhizobium*/*Azospirillum* had a significant effect (Table 3).

## 4. Discussion

## Search results

Interestingly, many articles were generated and not used in the analysis because of their keywords. *R. leguminosarum* was one of the microorganisms that was most commonly used as a treatment in the twenty-six articles (approximately 58%). A systematic review, as described in Table 1, revealed that cyanobacteria such as *Scytonema bohneri* and *Nostoc commune* are potential candidates for use as inoculants in the coinoculation of pea to increase seedling development after germination (Gayathri et al. 2017).

In the 1990s and early 2000s, a literature search revealed that *Rhizobium* inoculation may interact synergistically with the biocontrol of *Pythium* and other pathogens, causing dumping off, root rot disease, delayed germination and other effects on pea production in the field (King and Parke 1993; Hoflich and Ruppel 1994; Hwang et al. 1996; Wang et al. 2003; Mabrouk et al. 2007).

However, before employing plant growth-promoting rhizobacteria (PGPR) as coinoculants with root-nodulating rhizobia for a particular crop, it is essential to evaluate their potential adverse effects on other crops. From 2000 to 2010, the processes of 'inoculation,' '*Rhizobium*' and symbiosis efficiency for this crop ('pea' and '*Pisum*') were the significant connections among the search terms (Ballard et al. 2004). In recent years, researchers have reported the possible mechanisms by which PGPR in soil promote pea plant growth, for example, when inoculated with *Bacillus* spp., *Azotobacter chroococcum, P. fluorescens, Azospirillum* and *Agrobacterium tumefaciens* (Khalifa and Almalki 2015; Singh et al. 2019; Ejaz et al. 2020; Gupta et al. 2021; Shabaan et al. 2021).

Although information on inoculation studies with bacterial strains of PGPR and rhizobia is available (Jauhri et al. 1979; de Freitas et al. 1993; Kumar et al. 2001; Geneva et al. 2006; Egamberdiyeva 2008; Mishra et al. 2009; Mishra et al. 2012; Ahmad et al. 2013; Khalifa et al. 2016; Rapčan et al. 2017), these findings are scarce in the area of cyanobacterial inoculants for peas, and variety-specific responses have not been explored. The understanding that each plant variety exhibits a pattern of root exudates in response to elicitors in the soil is important for microbial inoculant development. However, other factors that affect the microbial population and activity of soil are essential nutrients such as phosphorus (Kumar et al. 2014). These authors highlight the role of arbuscular mycorrhizal fungi in economizing the inorganic phosphorus (P) and water needs of okra (*Abelmoschus esculentus*)–pea cropping in a sustainable system. Only a few articles have addressed the amount of nitrogen fixed by rhizobia and pea symbioses. We highlight a study in northern Ethiopia where the amount of nitrogen gained by legumes from BNF was an average of 23 kg N $ha^{-1}$ for field pea (*Pisum sativum*) and 32 kg N $ha^{-1}$ for dekeko (*Pisum sativum* var *abyssinicum*) (Mesfin et al. 2020).

The pea has a relatively high P requirement, which is necessary to promote extensive root systems and vigorous seedlings and to support excellent nodule results (Osman et al. 2010). The P content in the soil also plays an essential role in the BNF process. The BNF in acidic soils is greatly affected by the reduced number of rhizobia, e.g., for common bean, and the diversity was lower in acidic soil than in liming soils with a low aluminum content and high pH (Andrade et al. 2020a; Andrade et al. 2020b). According to Chaudhari et al (2020) pea plants shape their rhizosphere microbiome, possibly to meet its requirements for nutrient uptake and stress amelioration. Fungi grow well under acidic conditions; for example, arbuscular mycorrhizal fungi (AMF) are suitable for acidic soils. In this scenario, the use of *Rhizobium* and AMF in peas can be beneficial. A consortium of AMF enhances salt tolerance by maintaining ionic balance and the antioxidant enzyme system and regulating the synthesis of malondialdehyde and secondary metabolites(Parihar et al. 2020b). These symbionts benefit the soil–plant system in several ways, such as by improving crop quality and soil health through the production of various biomolecules such as enzymes, vitamins, antibiotics and hormones (Kumar et al. 2014). Thus, these bioinoculants also confer multiple benefits to plants, such as drought resistance (Bashir and Naz 2020; Belimov et al. 2020), disease tolerance, and P solubilization (Harrier and Watson 2003).

The rhizobia associated with legume roots provide N to plants through biological $N_2$ fixation. However, AMF increase the availability of P and other soil nutrients, such as N, potassium (K), Ca, copper (Cu) and zinc (Zn), which are otherwise low in acidic soils (Kumar et al. 2014). The mutual association of legumes with rhizobia and AMF is called tripartite symbiosis. Rhizobium provides N, while AMF provide P to host plants, which in turn contributes carbon (C) to plant growth and metabolism through carbohydrate/photosynthesis. Thus, coinoculation with *Rhizobium* and AMF can be beneficial for pea cultivation (Parihar et al. 2020a).

**A**

**B**

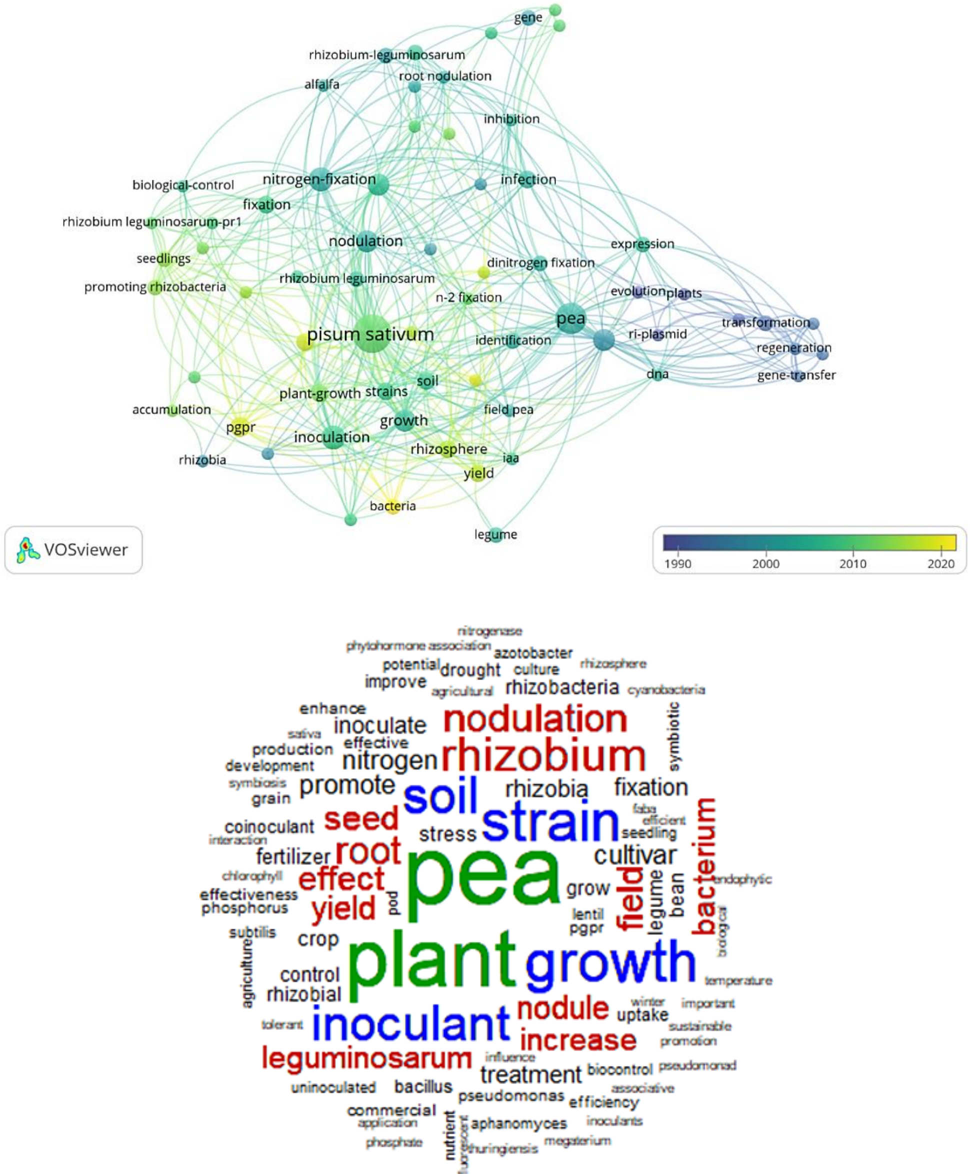



**Figure 2.** Density frequency showing the association with the nearby terms of the represented keywords (*Pisum sativum*) AND (inoculation) AND (strain) used in Web of Science® (WoS) database search from 1990 to April, 2022 (n=177). A - Illustration using WOSviewer. The larger the size of the bubbles the greater the association. B - Illustration using Iramuteq. The larger the size of the letters, the higher the frequency indicated by varying colors: green indicates most frequent term, blue denotes the second group of word frequency; red and black represent the less frequent terms.

## Analysis comparative of the grain yield

However, competitiveness for nodulation is likely established during early plant-bacterial interactions and/or bacterial colonization of the symbiotic organ but may not be directly related to the amount of N supplied through symbiosis. However, Bourion et al. (2018) proposed that both strain competitiveness and nitrogen fixation efficiency should be regarded as selection criteria for enhancing pea crop production. For example, in China, the nitrogen fixed by and grain yield of pea increased in response to inoculation with selected rhizobium strains (He et al. 2011).

Diazotrophic cyanobacteria species, which are commonly used in many crops according to studies that have reported their effects on soil carbon and N content (Prasanna et al. 2014b; Sousa et al. 2022) have been selected for use as inoculants. The production of the phytohormone indole-3-acetic acid (IAA) is widespread among bacteria that inhabit the rhizosphere of these plants. Diazotrophic and photosynthetic microorganisms, such as cyanobacteria, are also known to increase plant growth by producing IAA and improving soil structure through the secretion of extracellular polysaccharides that help soil aggregation (Boopathi et al. 2013; Prasanna et al. 2013).

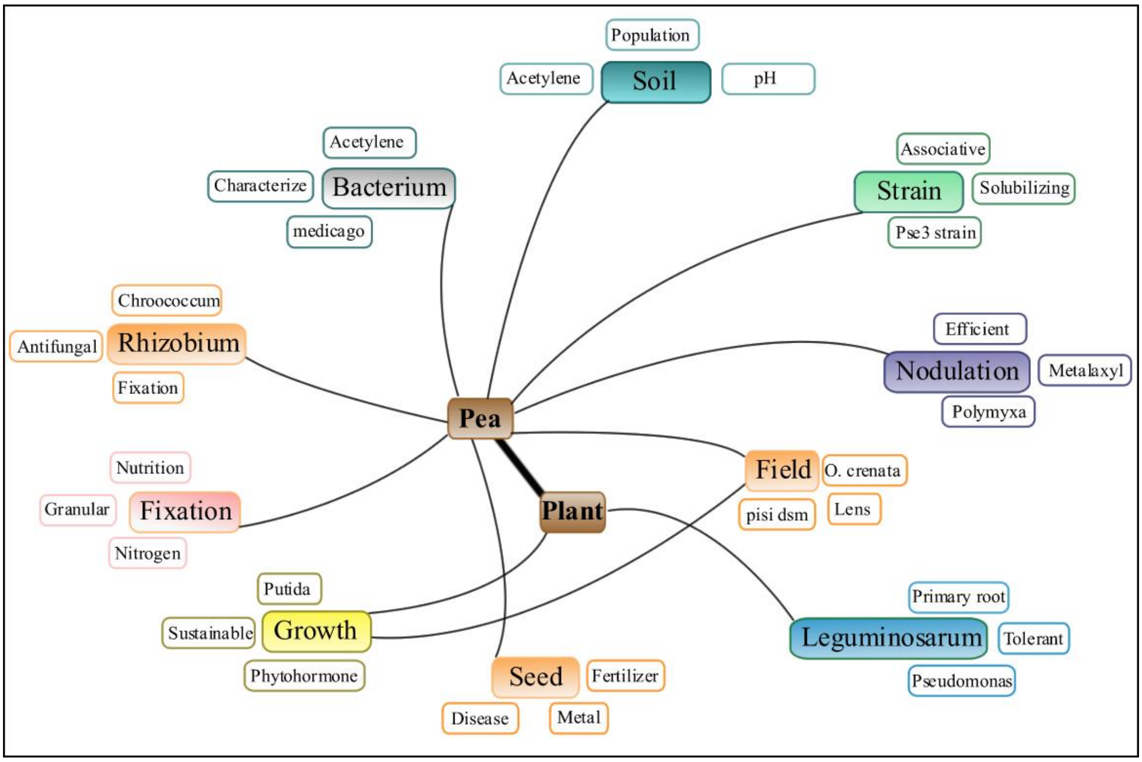



**Figure 3.** Similarity analysis of textual analysis of keywords used in the search of systematic review from 1990 to April, 2022 (n=177).

Interactions between bacteria and cyanobacteria have proven valuable as agents that promote plant growth and improve soil fertility, resulting in increased crop quality for wheat (Rana et al. 2012). In other studies, the role of strains of bacterial cyanobacteria in promoting growth in plants and new biofilms based on cyanobacteria has been established to increase soil fertility and the development of wheat, rice, tomato, cotton, corn and soybean plant (Prasanna et al. 2014a). Common bean had the highest yield when seeds were inoculated with *Anabaena cylindrica*, *Azospirillum brasilense* and a mixture of rhizobia (*Rhizobium freirei*+*R. tropici*) (Horácio et al. 2020). In maize, coinoculation of *Anabaena cylindrica* with *Azospirillum brasilense* increased the grain yield of maize hybrids (Gavilanes et al. 2020). A biofertilizer mixture (*Azotobacter chrocoocum*, arbuscular mycorrhizal fungi, and *Bacillus circulans*) with organic fertilizers enhanced maize growth and yield and nutrient uptake (Gao et al. 2020). In wheat, the consortium application of these strains resulted in a 17% increase in yield. It is evident from the results that the application of the consortium was more effective than the application of the single agent or coinoculation (Hussain et al. 2020).

**Table 3.** Descriptive statistical analysis of pea (*Pisum sativum*) grain yield (kg $ha^{-1}$) based on data from field-scale studies (n=85) from the published articles from 1990 to April 2022 (n=85) that present non-inoculated (Non-Inoc) versus inoculated (Inoc) with Rhizobium/Azospirillum treatments.

| | Non-inoc | Lower conf. | Upper conf. | Inoc | Lower conf. | Upper conf. |
|---|---|---|---|---|---|---|
| Min | 758 | | | 920 | | |
| Max | 5110 | | | 5800 | | |
| Mean | 2240 | 2010 | 2470 | 2887 | 2613 | 3149 |
| Std. error | 120 | 101 | 136 | 139 | 121 | 155 |
| Stand. dev | 1108 | 956 | 1278 | 1282 | 1135 | 1453 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Median | 2080 | 1690 | 2360 | 2770 | 2442 | 2950 |
| 25% percentile | 1350 | 1208 | 1500 | 1855 | 1300 | 2216 |
| 75% percentile | 3090 | 2870 | 3580 | 3623 | 2952 | 4052 |

With boostrap (N=9999).

Inoculants containing PGPR, which are known to have beneficial effects on the growth of legumes because they can produce several metabolites that are released, have been shown to increase legume growth and development under controlled and field conditions. These compounds indirectly or directly stimulate plant growth, and some strains have been shown to improve nodulation and nitrogen fixation by influencing the interaction between the plant and rhizobia (Yolcu et al. 2012).

Compared with no inoculation, inoculation with *Rhizobium* strains from lentil (*Lens culinaris* Medik.) grown on soils in the arid region of Pakistan showed potential for use as agents or biofertilizers for stimulating the growth and nutrient accumulation of lentil (Zafar et al. 2012). Inoculation with new rhizobia significantly enhanced the growth, nutrient acquisition (Moreno-Chirinos et al. 2016) and grain yield of pea plants· (Arafa et al. 2018).

Plant growth-promoting rhizobacteria colonize the rhizosphere and are thought to directly or indirectly improve plant growth via various mechanisms. Due to their rapid colonization and stimulation of plant rhizosphere growth, most PGPR have gained importance as one of the principal components for improving the yield of several different agronomic crops, e.g., wheat (*Triticum aestivum*), rice (*Oriza sativa*) and black gram (*Vigna mungo*) (Mader et al. 2011) rice (Ríos-Ruiz et al. 2020). Specifically, due to the increase in the absorption of nutrients by plants, BNF, the production of siderophores and phosphate solubilization are considered the main mechanisms of mediation responses (Dey et al. 2004) or biocontrol (Lugtenberg and Kamilova 2009) and rapid nodulation. (Lira Júnior et al. 2005).

Peas are rich in fiber, protein, vitamins, minerals and lutein. They exert beneficial prebiotic effects on the large intestine and produce various phytochemicals with antioxidant and anticarcinogenic activities (Dahl et al. 2012). In Brazil, some farmers produce peas, mainly pods, as an alternative for rotating tomato (*Lycopersicon esculentum*) crops; as a sustainable alternative to avoid nitrogen fertilizers, this type of technology improves soil quality. Therefore, microbial inoculants are recommended for use in pea production systems because they aim to maintain fertility and increase sustainability by maintaining the biological health of the soil.

Although there are a large number of field studies highlighting the increase in pea grain production due to inoculation with *R. leguminosarum* (Begum et al. 2001; Kutcher et al. 2002; Clayton et al. 2004; Slattery et al. 2004; Rabbani et al. 2005; Ahmed et al. 2007; Uher et al. 2010; Huang et al. 2017; Muniz et al. 2017; Almaca et al. 2021), it is still important to identify inoculation biotechnologies for application in agricultural production fields. In the search for commercial inoculants, it is necessary to prioritize stable symbiotic plasmids, aiming for commercial inoculant recommendations. In soil with a history of previous *Rhizobium* inoculation, Chemining'wa and Vessey (2006) suggested that there is a decrease in the effectiveness of the inoculant strain after several years in the soil. In Brazil, a selected strain of *R. leguminosarum* (SEMIA3007) recommended for commercial pea inoculation has lost the capacity for nodulation.

## 5. Conclusions

There is potential for the inoculation and/or coinoculation of *P. sativum* with nitrogen-fixing bacteria/plant growth-promoting rhizobacteria. The inoculation/coinoculation of peas promotes a greater yield than that of noninoculated plants due to biological $N_2$ fixation, which depends on the soil moisture and fertility, pea cultivar and *Rhizobium* strains. There is a great opportunity for improving the response of pea to symbiosis by both using selected efficient strains of *Rhizobium* to inoculate and improving whole soil nutrients and crop management. Studies are still needed to achieve the goals of supplying nitrogen via biological fixation by plant and *Rhizobium* symbiosis to improve the agronomic performance of pea to a substantial extent.

**Authors' Contributions:** All authors contributed equally to the study conception and design: material preparation, data collection, and writing of the manuscript. All authors have reviewed and approved the manuscript for publication.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethics Approval:** Not applicable.

**Acknowledgments:** HAMV acknowledges a scholarship from the National Council for the Improvement of Higher Education (CAPES, 001). This study was partially supported by the INCT-CNPq (Brazilian National Council for Scientific and Technological Development) (MPCPAgro 465133/2014-2). HFA acknowledges UNIFIL to the partial support of the Extension project: "Use of safe biological resources and phytosanitary techniques for conservation agriculture". DSA and is also research fellow of CNPq (315060/2020-4).

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**Received:** 08 November 2022 | **Accepted:** 13 June 2024 | **Published:** 18 July 2024