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META-ANALYSIS

Effects of *Rhizobium* inoculum compared with mineral nitrogen fertilizer on nodulation and seed yield of common bean. A meta-analysis

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Abstract
Common bean (*Phaseolus vulgaris*) is one of the most important legumes for human consumption. It is highly adaptable to different edaphoclimatic conditions, being an important crop in addressing global food security challenges. The common bean production segment has undergone an intense technological advance, with a focus on the use of technologies to increase the availability of nitrogen (N) and the crops' seed yield, while enhancing economic and ecological sustainability. Based on this, the present meta-analysis aimed to evaluate the effects of *Rhizobium* inoculation (RI), in comparison with mineral-N fertilization (NF), on the main nodulation characteristics, yield components, and seed yield of common beans. This study represents the largest assessment yet on this topic. We used data from peer-reviewed publications and, after extensive bibliographic research, analyzed 68 studies from seven countries. We found that RI increased seed yield (32.96%) but not to the same extent as NF. The RI is on average 12.31% less efficient than NF; however, when we categorized the factors, such as the time of year when common beans were grown, the soil management system, and the soil physicochemical characteristics, the RI effects were more promising. Here we show for the first time that RI was more efficient than NF when common beans were cultivated in the dry season, under a no-tillage system, and in soils with high organic matter content, with a potentially positive impact on yields. In addition, the difference in the efficiencies of RI and NF was attenuated when common beans were grown in soils with a clay texture, eutrophic, with low to neutral acidity, and with an adequate phosphorus availability, and using at least 10 g of rhizobial inoculum per kg of seeds.

Keywords Sustainable agriculture · Inorganic fertilization · Biological nitrogen fixation · Symbiotic interaction · *Phaseolus vulgaris*

1 Introduction

Common beans (*Phaseolus vulgaris* L.) can be considered an ally in addressing global food security challenges. Its seeds are used as a primary source of protein, dietary fiber, and energy (starch), especially in less developed countries (Araujo et al. 2020; Los et al. 2018). Amongst the bean species cultivated worldwide, common beans are unique due to their great variations in the color of the seed coat and the characteristics of the pod (Allen 2013; Sinkovič et al. 2019).

The total bean cultivated area and production from 104 countries in 2019 were 33 million hectares and 29 million

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tons, respectively with an average seed yield of 1557 kg ha^{-1}. The 10 largest world producers of beans, in descending order, are Myanmar, India, Brazil, China, Tanzania, Uganda, USA, Mexico, Kenya, and Burundi. These countries collectively contribute to 70.8% of the total amount of beans produced in the world, corresponding to 20.5 million tons (FAO 2021).

In addition to its worldwide importance, the common bean is capable of growing under different soil conditions and climatic variations (Shamseldin and Velázquez 2020). The crop is economically important due to its cultivation at all technological levels of production. The majority of the producers of this crop are smallholder growers, who have limited resources for seed production (Castro-Guerrero et al. 2016; Wanjala et al. 2019). In addition, the legume has a high requirement for nitrogen (N) (Maia et al. 2017; Soratto et al. 2013; Jena et al. 2022). The average export of nutrients is about 35.5 kg of N per ton of seed produced, with greater demand during flowering and seed filling (Soratto et al. 2013). Therefore, N deficiency is one of the main factors limiting common bean yield (Maia et al. 2017; Soratto et al. 2014).

Common bean production value chains have undergone a paradigm transformation in the last three decades, which has promoted a significant increase in seed yield (Nassary et al. 2020). In particular, cultivation techniques such as the use of mineral-N fertilization (NF) have been widely adopted. Fertilizer application results in taller plants with greater above-ground biomass and dry matter yields in comparison with plants grown in the absence of NF (Soratto et al. 2014). In addition, fertilizer user results in a linear increase in the seed yield of common bean crops up to maximum rates of 180 kg N ha^{-1} (Deresa 2018; Mingotte et al. 2019; Soratto et al. 2017). Despite its recurrent use, N is easily lost through leaching, volatilization, and denitrification (Nyawade et al. 2020). These potential losses represent high economic and environmental costs associated with NF (Figueiredo et al., 2016; Silva et al. 2020). Consequently, there is debate about the adoption of more sustainable and economically efficient agricultural techniques.

Sustainable agriculture is increasingly being recognized as a tool to address the aforementioned challenges. It focuses on the use of technologies that aim at increasing the availability of N and the productivity of legumes, emphasizing the economic and ecological aspects of production systems (Araujo et al. 2020; Raza et al. 2021; Paudyal and Gupta 2018; Soratto et al. 2022). An example of this is biological N fixation (BNF), which can be an alternative for the supply of N, reducing the use of N fertilizers and, consequently, minimizing the environmental impacts of N leaching in rivers and lakes (Figure 1) (Shibata et al. 2017).

Symbiotic associations are estimated to be able to fix up to 80% of the N requirement in agricultural areas (Herridge et al. 2008; Mendoza-Suárez et al. 2020). However, the total or partial replacement of NF by *Rhizobium* inoculation (RI) techniques in common bean crops is still very irresolute due to variations in its impact on seed yield (Hungria et al., 2013). Herridge et al. (2008), while using the method of the percentage of N derived from atmosphere (%Ndfa), found that the food legumes in the global area can fix about 2.95 Tg of N $year^{-1}$. Among the crops, the authors highlighted are chickpea (*Cicer arietinum* L.) (0.60 Tg), common beans (0.58 Tg), pea (*Pisum sativum* L.) (0.57 Tg), and other pulses (0.47 Tg) are the predominant contributors. However, compared with the oilseed legumes, the food legumes have less contribution. For instance, soybean [*Glycine max* (L.) Merr.] and groundnut (*Arachis hypogaea* L.), together contribute 6.3 times higher BNF annually (18.5 Tg $year^{-1}$) (Rao and Balachandar 2017).

Therefore, a better understanding of the factors that limit common bean seed yield under RI is important, as this information will serve to support technical management decisions based on scientific evidence (Pittelkow et al. 2015). Considering the growing interest and encouragement of inoculation techniques with *Rhizobium* bacteria as a tool to mitigate the harmful effects of chemical fertilization on the environment, we used this meta-analysis to synthesize scientific evidence of the effects of RI, compared to those of NF, on the main attributes of a common bean plant and its seed yield at a global level.

The objectives of this study were: (i) to evaluate the current state of inoculation with bacteria of the genus *Rhizobium* in common beans globally; (ii) verify the effects of RI, as well as compare them with those of NF, on the nodulation characteristics, yield components, and seed yield of common beans; and (iii) identify the factors that affect the efficiency of RI in the common bean crop, in comparison with the efficiency of NF.

2 Materials and methods

2.1 Systematic review and data selection

The selected papers were published between January 2000 and January 2021. Studies prior to the defined minimum year were excluded because they did not meet the inclusion criteria that we will discuss. Data from articles and scientific notes published in scientific journals were collected through the bibliographic review using the following databases: Web of Science (https://webofknowledge.com/), SCOPUS (https://www.scopus.com/), SciELO (https://scielo.org/), and Google Scholar (https://scholar.google.com/).

As a search strategy, the following keyword combinations were used: “Phaseolus vulgaris OR common beans”, “inoculation with Rhizobium OR inoculation of rhizobia”, “nitrogen fertilization OR fertilization N mineral”, and “grain yield OR seed yield”. We sought to include studies that demonstrated the response of common beans, in terms of seed yield (main

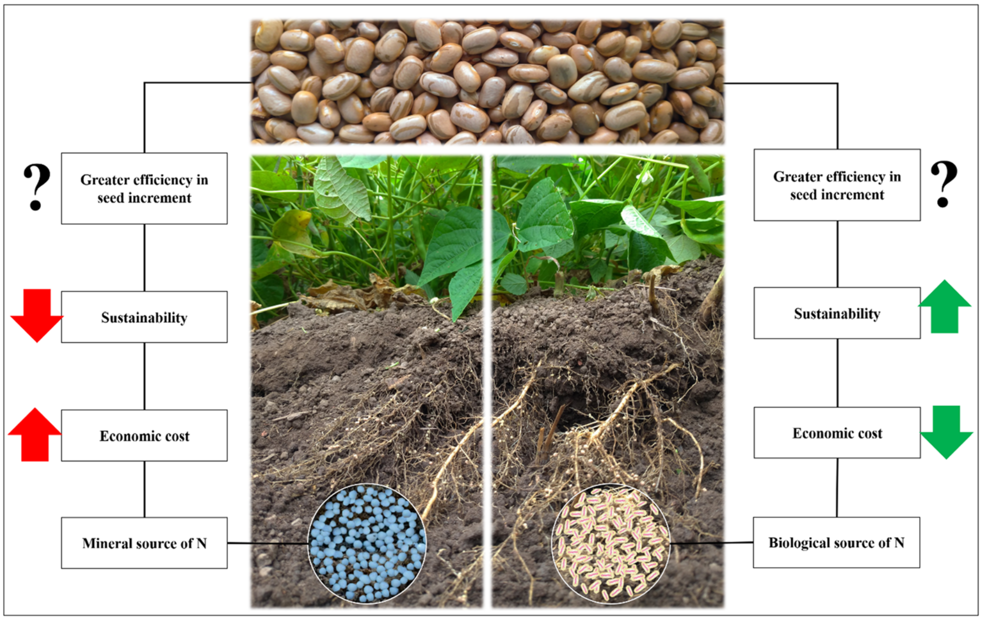

Fig. 1 Main sources of N supply for common beans and positive and negative aspects of both techniques: fertilization with mineral N (left) and biological N fixation through diazotrophic bacteria (right). Photo credit: Westefann Sousa.

variable), to RI and NF. When the studies presented the results for a control treatment (CK), that is, without RI and NF, these data were also compiled. When possible, the results of nodulation and yield components of common beans (secondary variables) were also compiled as a function of the treatments. The secondary variables selected were the number of nodules per plant, nodule dry mass per plant in grams, number of pods per plant, number of seeds per pod, and 100-seed weight in grams.

2.2 Inclusion and exclusion of studies

A detailed review protocol was developed with some inclusion and exclusion criteria, which were applied to select the studies found in the databases:

a) Experiments that presented treatments with RI and NF were included. The treatments with RI and NF should be the factors studied in isolation. Studies were excluded when it was not possible to isolate the effects of RI and NF on the characteristics of the common bean crop.
b) No studies were selected that showed only the effect of one treatment (RI or NF) on the characteristics of common beans. In addition to the studies having to present both treatments, agronomic practices were to be similar between the plots with RI and NF.
c) Studies that demonstrated the effects of treatments on seed yield and other evaluated characteristics of common beans were included. Nonetheless, when the study did not show the effect of treatments on seed yield, but rather showed only the results of the other characteristics of common beans, these were also excluded.
d) Studies that were conducted exclusively under field conditions and that presented the number of repetitions used to calculate the mean were included in the meta-analysis.
e) The search strategy partly entailed studies conducted in a single season (growing season) and/or location (environment) were excluded. Other studies that were exceptionally accepted included those that had tested different cultivars and/or strains of *Rhizobium*, even though they were conducted in a single season and/or place. In this case, studies were included if they demonstrated the reliability of the results through measures of variability, such as standard deviation and coefficient of variation.

For the results of the studies that were presented in figures, such as bar or line graphs, the images were processed in the Web Plot Digitizer software (version 4.4; https://automeris.io/WebPlotDigitizer/) to extract the underlying numerical data and visualize the results.

2.3 Abstraction and organization of data

A selected sample was evaluated by performing a full reading of the entire work. After the second screening, the studies were submitted to data abstraction using a spreadsheet editor, in which all the information of the studies believed to be important was described. This step was performed manually, and each study was processed individually (Ahn and Kang 2018).

The information considered important for the present study, in addition to the variables evaluated included the time of year when the common bean was grown (growing season), Köppen-Geiger climatic classification of the region where the experiment was conducted (climate), classification of the soil

(soil type) based on the United States Department of Agriculture (United States Department of Agriculture (USDA), 2014). The soil-related information was based on the soil management system used in the experiment (soil management), percentage of clay in the soil (soil clay content), base saturation in the soil (BS), soil organic matter content in g dm^{-3} (OM), soil hydrogen potential (pH), and soil available P (in mg P dm^{-3}). With regard to agronomic management of the crop, we considered inoculant dose in g kg^{-1} of seeds (inoculant dose), number of strains in the composition of the inoculant (number of strains), type of strain used in the inoculant (strain type), rates of P fertilization in kg P_2O_5 ha^{-1} (P fertilization), and rates of NF in kg N ha^{-1} (N fertilization). Information on soil characteristics was selected from samples extracted at a depth of 0–20 cm from the soil. After all the studies were carefully selected, the information was stratified into 14 categories, as performed by Peixoto et al. (2020), and adapted for the present study (Table 1).

Commercial rhizobia strains, categorized in the meta-analysis, are those intended for the manufacture of inoculants, whose efficiency of these strains is already known. The strains called isolates are those under study, that is, rhizobia isolate that are characterized and subjected to experiments to validate their efficiency in the BNF process. Further information on the various strains and amounts of inoculant used by various studies and the soil conditions tested is provided in the supplemental material (Supplementary Table S1). The source used for NF was not considered as a categorical factor, given that the number of observations was higher for urea, with 791 of the total number of observations (96.94%), followed by ammonium nitrate with 17 observations (2.08%) and ammonium sulfate with eight observations (0.98%). Thus, the meta-analysis proceeded without categorizing the different sources used for the NF.

Table 1 Categories and their respective stratifications (classes) used in this study.

Categories	Classes
Growing season	Winter, Rainy season, and Dry season
Climate	Tropical, Semi-arid, and Temperate
Soil type	Oxisol, Ultisol, and Entisol
Soil management	Conventional and No-tillage
Soil clay content (%)	≤30, 30-60, and ≥60
Soil base saturation (%)	≤50, 50-70, and ≥70
Soil organic matter (g dm^{-3})	≤25 and 25–50
Soil hydrogen potential (pH)	≤5, 5-7, and ≥7
Soil available P (mg dm^{-3})	≤15 and >15
Inoculant dose (g kg^{-1} of seeds)	<10, 10, and 100
Number of strains	1 and >1
Strain type	Commercial and Isolated
P fertilization (kg P_2O_5 ha^{-1})	≤30, 30-60, 60-90, and ≥100
N fertilization (kg N ha^{-1})	≤40, 40-60, 80-100, and ≥120

2.4 Data analysis

The response variable used in the present meta-analysis, called the response ratio (*RR*), was calculated using the natural logarithm of the response ratio (ln*RR*) (Equations 1a, b, and c), comparing the measures of effects (ME) of the treatments. Initially, the effects of RI vs CK, NF vs CK, and RI vs NF on the nodulation characteristics, yield components, and seed yield of common beans were compared. Then, the effects of RI vs NF treatments were compared within the categories defined in Table 1.

$$(\text{RI vs CK})\ \ln RR = \ln\frac{\mathrm{ME_{RI}}}{\mathrm{ME_{CK}}} \tag{1a}$$

$$(\text{NF vs CK})\ \ln RR = \ln\frac{\mathrm{ME_{NF}}}{\mathrm{ME_{CK}}} \tag{1b}$$

$$(\text{RI vs NF})\ \ln RR = \ln\frac{\mathrm{ME_{RI}}}{\mathrm{ME_{NF}}} \tag{1c}$$

where *ln* is the natural logarithm, $\mathrm{ME_{RI}}$ is the measure of the effect of inoculation with *Rhizobium*, $\mathrm{ME_{NF}}$ is the measure of the effect of treatment with NF, and $\mathrm{ME_{CK}}$ the measure of the effect of control treatment.

A few studies have presented measures of variation. Thus, we chose to weight (*w*) the individual observations of each study based on the number of repetitions of the experiment (Equation 2a, b, and c) (Pittelkow et al. 2014).

$$(\text{RI vs CK})\ \mathrm{w} = \frac{\mathrm{n_{RI}} \times \mathrm{n_{CK}}}{\mathrm{n_{RI}} + \mathrm{n_{CK}}} \tag{2a}$$

$$(\text{NF vs CK})\ \mathrm{w} = \frac{\mathrm{n_{NF}} \times \mathrm{n_{CK}}}{\mathrm{n_{NF}} + \mathrm{n_{CK}}} \tag{2b}$$

$$(\text{RI vs NF})\ \mathrm{w} = \frac{\mathrm{n_{RI}} \times \mathrm{n_{NF}}}{\mathrm{n_{RI}} + \mathrm{n_{NF}}} \tag{2c}$$

where $\mathrm{n_{RI}}$ is the number of repetitions for RI, $\mathrm{n_{NF}}$ is the number of repetitions for NF, and $\mathrm{n_{CK}}$ is the number of repetitions of CK.

To evaluate the statistical variability of the sampled data, the confidence intervals were calculated using the bootstrap method with a 95% probability. This procedure is based on the creation of new samples, or subsamples, of the same size as the initial sample through resampling with replacement (Bishara and Hittner 2016; Michalak et al. 2002). Thus, confidence intervals were generated for ln*RR* using 5000 bootstrap interactions. To eliminate abnormal observations in the data set, which would greatly affect the confidence interval estimate, observations with an ln*RR* standard deviation greater than five were excluded (Peixoto et al. 2020; Pittelkow et al. 2014).

Bootstrap resampling was stratified in each category, as pre-established in Section 2.3., for the main variable (seed yield). For the secondary variables (nodulation and yield components), the analysis proceeded in general, not considering the categories. We proceeded in this way because the number of observations for the secondary variables was lower than that for the main variable, in addition to the data of the secondary variables not encompassing all categories. The results were considered significant when bootstrap confidence intervals did not overlap to zero (Wood 2004).

To facilitate the discussion of the data, all the results of the variables were reported as the percentage variation of the treatment RI vs CK, NF vs CK, and RI vs NF, using Equation 3 (Peixoto et al. 2020):

$$RR(\%) = \left(\exp^{\ln RR} - 1\right) \times 100 \qquad (3)$$

where $RR(\%)$ is the percentage of the response ratio and $\exp^{\ln RR}$ is the exponent of the natural logarithm of the response ratio.

All analyses were processed using R software version 4.0.3 (R Core Team 2020), and the following packages were used: tidyverse, readxl, ggeffects, boot, and broom (Canty and Ripley 2020; Lüdecke 2018; Robinson et al., 2020; Wickham and Bryan 2019; Wickham et al. 2019).

3 Results and discussion

3.1 Overview

The search in the databases using the descriptors (keywords) resulted in a total of 1290 studies. With the application of the inclusion and exclusion criteria, the number of selected studies was reduced to 68, which was then processed in the meta-analysis. From the selected studies, 758 observations were extracted (a database was provided as a supplementary document containing all data extracted from the studies). The language used in the publication of the largest number of studies was English (60%; n = 41), followed by Portuguese (37%; n = 25) and Spanish (3%; n = 2). A greater number of studies were conducted in Brazil (76.47%; n = 52), followed by Ethiopia (10.29%; n = 7), Iran (4.41%; n = 3), Spain (2.94 %; n = 2), Turkey (2.94%; n = 2), Tanzania (1.47%; n = 1), and Peru (1.47%; n = 1) (Figure 2). In Brazil, almost all studies were concentrated in the Southeast, South, and Midwest regions of the country.

3.2 Effects of Rhizobium inoculation on nodulation characteristics and yield components, in comparison with those of mineral-nitrogen fertilization

Overall, RI increased nodulation characteristics compared to the control treatment (CK), with an effect size in the order of 19.04% and 37.03% for the number of nodules per plant and nodule dry mass per plant, respectively (Figure 3a and d). Even with the vast majority of soils sown with common beans containing indigenous rhizobia that can interfere with the establishment of inoculated strains (Vargas et al., 2000), these results demonstrate the efficiency of inoculants outweighed the competition with native rhizobia, without major interference in BNF. As for NF, compared to CK, there was a negative effect on the number of nodules per plant and nodule dry mass per plant, with respective reductions of 73.08% and 88.46% (Figure 3b and e). The inhibition of the microbial activity of rhizobia by NF is already well known in the literature (Glodowska and Wozniak 2019), which explains this result in terms of nodulation. Regarding the compared effects of RI and NF, there was an increase in nodulation characteristics for RI compared to NF (Figure 3c and f). This increase was in the order of 72.49% and 43.06% for the number of nodules per plant and nodule dry mass per plant, respectively.

Common beans are believed to be an inefficient fixer of atmospheric N (N_2), due to factors, such as the genetic characteristics of the other symbiotic partners, as well as the soil and environmental conditions (Yadegari et al. 2010). In addition, promiscuity with a wide range of indigenous rhizobia in the soil becomes a barrier to the symbiotic effectiveness of the legume with more efficient rhizobia (Shamseldin and Velázquez 2020; Zinga et al., 2017). In the present study, the nodulative aspects of the common beans were benefited by RI, in comparison with those with NF and/or CK, which proves the greater responsiveness of common beans to symbiosis with N-fixing bacteria of the *Rhizobium* genus and greater efficiency of inoculants introduced in the crop cultivation system.

The increase in nodulation of common beans through RI provides greater efficiency for BNF (Hungria et al., 2013), as the N input required by the legume can be supplied, in part, by the RI. In addition, high NF rates considerably reduce the nodulation of common beans and, consequently, can suppress the expression of desirable characteristics associated with BNF, such as nitrogenase activity (Müller et al. 1993; Reinprecht et al. 2020; Soares et al., 2016), which is consistent with the results of the present study for nodulation variables.

As for the yield components, there were significant differences when comparing the effects of RI vs CK and NF vs CK (Figure 4). The increases in the number of pods per plant, number of seeds per pod, and 100-seed weight were 9.75%,

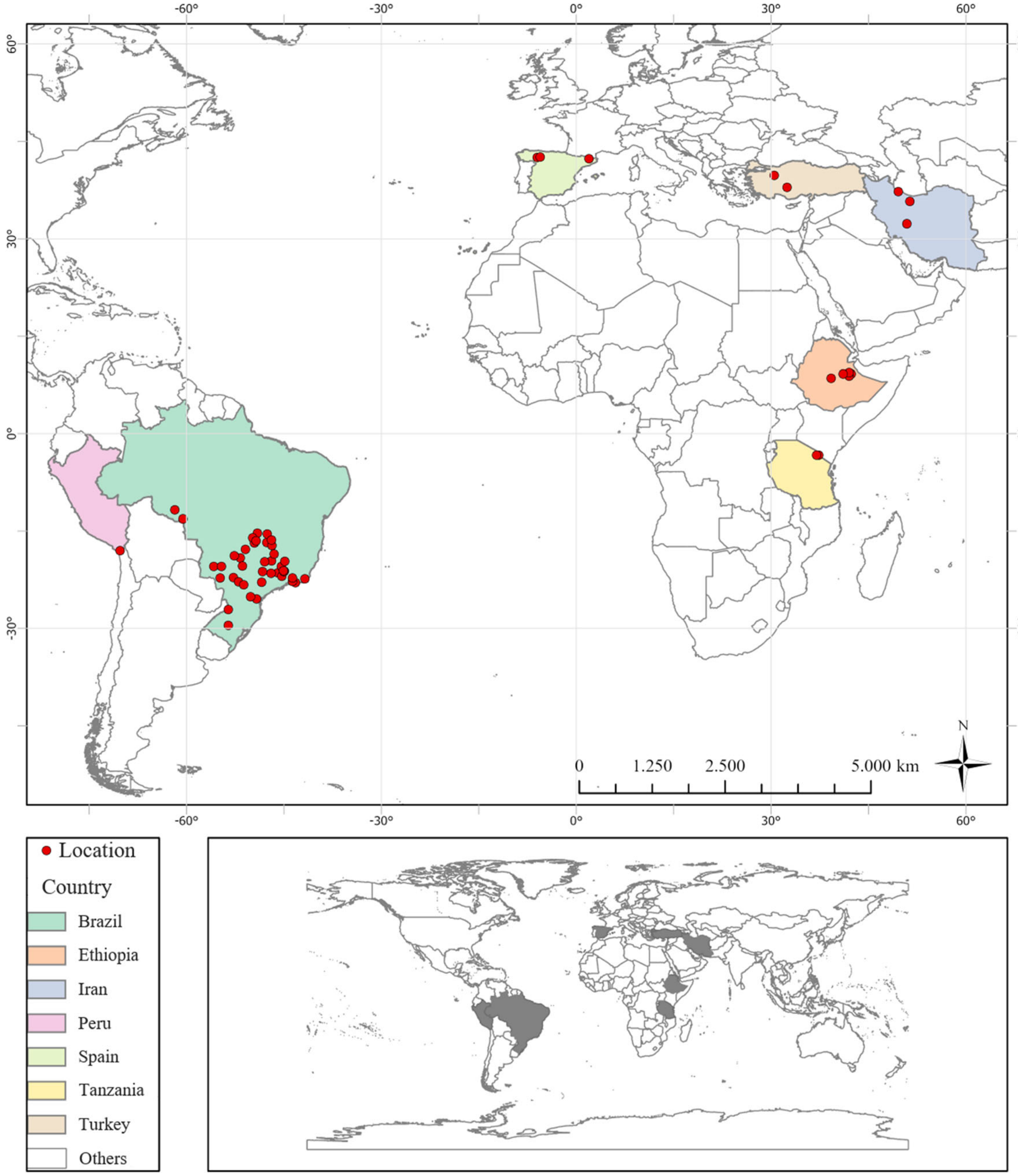

Fig. 2 Location of studies used in the meta-analysis that include comparisons between *Rhizobium* inoculation and mineral-N fertilization in common bean crops.

9.41%, and 8.83%, respectively, in the RI treatment compared to the CK (Figure 4a, d, and g). Compared to CK, the NF treatment increased the number of pods per plant, the number of seeds per pod, and 100-seed weight by 52.52%, 7.87%, and 9.02%, respectively (Figure 4b, e, and h).

The comparison between RI vs NF showed a reduction in the number of pods per plant (17.14%) in the RI treatment (Figure 4a). The number of seeds per pod and 100-seed weight showed no statistical difference between the RI and NF treatments (Figure 4b and c). These results indicate that RI has a similar effect to NF on the number of seeds per pod and 100-seed weight of common bean, but a much smaller effect on the yield component that is most affected by the availability of N, i.e., the number of pods per plant (Soratto et al. 2014, 2017). The greater availability of N promoted by NF, especially at the beginning of the cycle, probably stimulated greater vegetative growth of common bean plants. Larger plants with a greater number of branches produce a greater number of reproductive structures (Soratto et al. 2014). In addition, it was not possible to relate the greater nodulation capacity of the plant with the number of pods per plant (Figures 3 and 4).

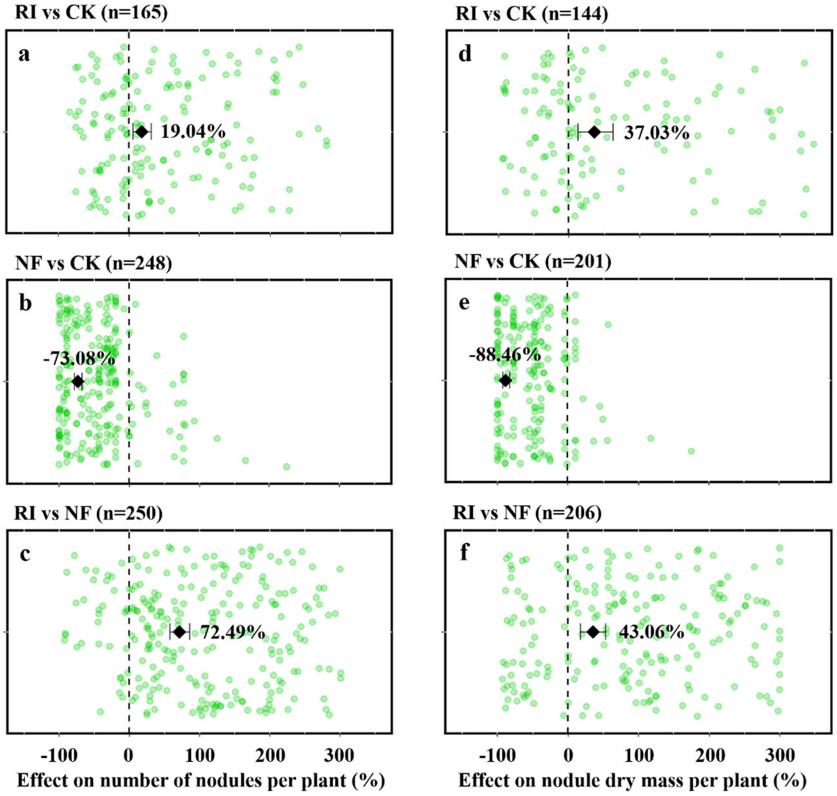

Fig. 3 Effects of Rhizobium inoculation compared to control treatment (RI vs CK, **a** and **d**), mineral-N fertilization compared to control treatment (NF vs CK, **b** and **e**), and Rhizobium inoculation compared to mineral-N fertilization (RI vs NF, **c** and **f**) on the number of nodules per plant (**a**, **b** and **c**) and nodule dry mass per plant (**d**, **e**, and **f**) of common bean crop. The green dots depict the observations of the sampled studies and the values in parentheses represent the sample size.

Previous studies have demonstrated that NF has a significant effect on the loading of common bean plants (Chekanai et al. 2018; Fageria et al. 2014a); however, the bean's response may vary according to the cultivar and environmental factors (Chekanai et al. 2018). This suggests that some yield components are much more subject to being controlled by potential common bean genes, as well as the influence of environmental conditions than by the RI and/or NF itself (Fageria et al. 2014b; Morad et al. 2013; Soares et al., 2016). The small variation observed in the effect of both treatments (RI and NF) on the number of seeds per pod and the 100-seed weight demonstrated this.

3.3 Effects of Rhizobium inoculation on seed yield compared to those of mineral-nitrogen fertilization

The overall seed yield response to RI and NF, both compared to CK, was significant, with an increase of 32.96% for RI (Figure 5a) and 46.69% for NF (Figure 5b). It is important to highlight that some farmers, mainly in developing countries, do not apply mineral-N fertilizers but rather depend totally on the residual supply of N from the soil and BNF. Ultimately, this makes the latter a cheaper and more sustainable alternative (Samago et al. 2018). Thus, the increase of 33% in common bean seed yield in the presence of RI, in relation to CK treatment, can guarantee the success of the crop in smallholder agricultural systems where farmers depend totally on this biological input. The opposite effect to that reported was found when comparing RI vs NF, in which RI had a 12.31% lower seed yield compared to NF (Figure 5c). This is a reflex of the smaller effect RI has on the number of pods per plant, compared to NF (Figure 4).

3.3.1 Climatic characteristics, management, and soil type

For cultivation in the dry season, RI showed a 5.76% increase in the seed yield, in comparison with NF (Figure 6a). In the cultivation carried out in winter and in the rainy seasons, there was a lower seed yield for RI compared with NF, with a difference of 11.88% and 9.42%. As for the results for the cultivation in the dry season, it is noteworthy that of all the observations compiled to reach this result (n = 61), they correspond to eight studies conducted without the use of irrigation, with well-distributed rainfall during the cultivation of common bean and without occurrences of water deficit. Previous research has demonstrated that plant growth-promoting rhizobacteria are more effective under drought conditions, which leads to a reduction in the use of NF (Barros et al., 2018; Rubin et al. 2017). It is also worth noting that in the period of drought cultivation, NF suffers a greater loss of N due to the increase in asynchronicity between the release of N through mineralization and the uptake of N by the crop (Ullah et al. 2020), which makes RI practices more efficient. It is also important to mention that, especially in the Central-

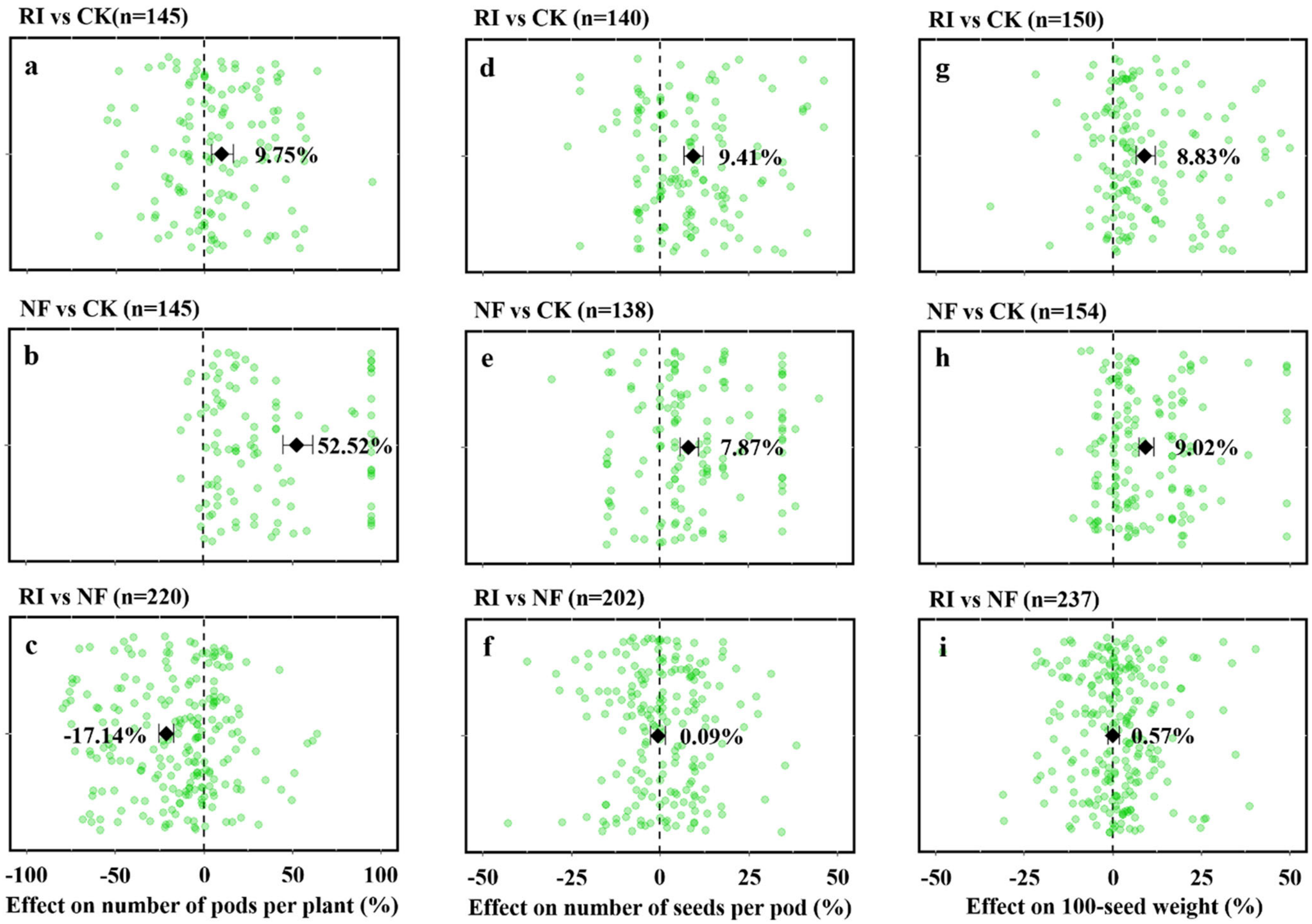

Fig. 4 Effects of *Rhizobium* inoculation compared to control treatment (RI vs CK), mineral-N fertilization compared to control treatment (NF vs CK), and *Rhizobium* inoculation compared to mineral-N fertilization (RI vs NF) on number of pods per plant (**a**, **b** and **c**), number of seeds per pod (**d**, **e**, and **f**), and 100-seed weight (**g**, **h** and **i**) of common bean crop. The green dots depict the observations of the sampled studies and the values in parentheses represent the sample size.

South region of Brazil, where most studies were conducted, the dry season (also called the second growing season, with sowing between January and March), normally has a higher occurrence of rain, especially in the early stage of the crop cycle, than in the winter and rainy seasons (CONAB 2021). The better-distributed rain at the beginning of the common bean cycle probably promoted a suitable establishment of the plant-rhizobia symbiosis. In this way, under the aforementioned conditions, we suggest that in the cultivation during the dry season, the farmer can invest exclusively in RI to supply N, which can become more efficient than fertilization with mineral N, with a significant increase in the common bean yield.

Regarding the climatic classification of the region, seed yield in the tropical (-11.81%) and semi-arid (-16.14%) climates were lower for RI compared with NF. Furthermore, in the temperate climate, the seed yield with RI was similar to that with NF (Figure 6b). In the plant-soil ecosystem, the temperature is an important environmental factor that influences the different interactions that occur between plants, soil, and microorganisms. This temperature effect can be indirect through its interaction with other environmental parameters, such as humidity or oxygen, or direct because it affects the rate of biological reactions (Prévost et al. 1999). The ideal growth temperature for most rhizobia is between 25 °C and 30 °C; however, the rhizobia survival in the soil and the symbiotic properties of *Rhizobium* strains are more affected by high temperatures, particularly under humid conditions, than by low temperatures (Pinto et al. 1998; Prévost et al. 1999). This may explain the similar effect of RI, in relation to that of NF, on the common bean yield in a region with a colder climate (temperate). It is commonly reported in the literature that the influence of environmental factors, with more favorable conditions of soil temperature and humidity, provide greater efficiency of BNF (Deak et al. 2019; Onwuka and Mang 2018; Sánchez et al. 2014). The contrary can also be true, especially in adverse environments, such as growing conditions in drought and temperate climatic zones. The

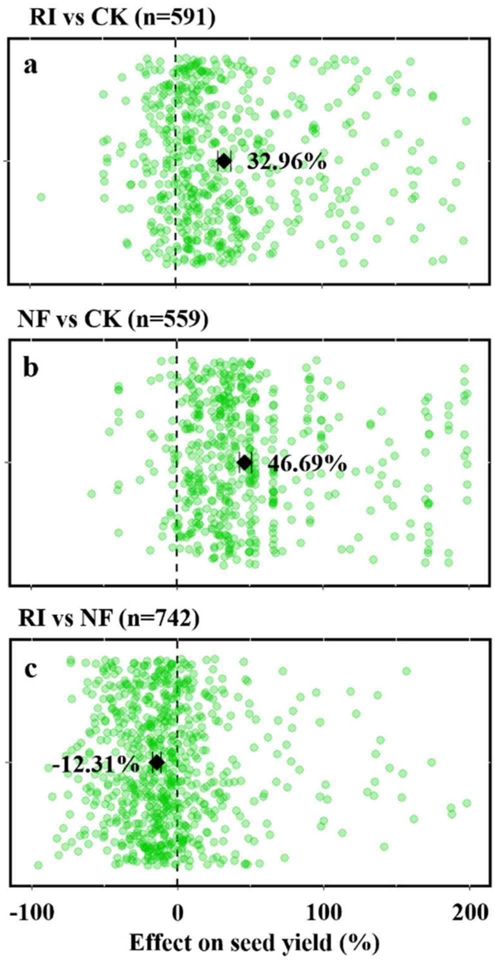

Fig. 5 Effects of *Rhizobium* inoculation compared to control treatment (RI vs CK, **a**), mineral-N fertilization compared to control treatment (NF vs CK, **b**), and *Rhizobium* inoculation compared to mineral-N fertilization (RI vs NF, **c**) on common bean seed yield. The green dots depict the observations of the sampled studies and the values in parentheses represent the sample size.

results of this study indicate the potential use of RI in contrasting environmental conditions, whether in low humidity or cold weather, and this should be used as a reference for future studies aimed at the management of BNF in common beans, assuming that these factors affect variations in the RI depending on the context analyzed.

The effects of RI, compared with those of NF, negatively interfered with the common bean seed yield in soils of the Ultisol and Entisol types, with a negative impact of 5.03% and 11.87%, respectively. However, RI increased the seed yield compared with that with NF when common beans were grown in Oxisol, with a 19.09% increase in the seed yield (Figure 6c). Another important category in this result was the soil management system used, with opposite results for the two types of systems, in which the seed yield response to RI was significantly lower (11.20%) when using conventional management, while the RI increased the seed yield by 5.41% in the no-tillage system, compared with those of NF, (Figure 6d).

For Oxisols, it is not possible to state that this alone is a major factor that benefits BNF in common beans, as this requires more detailed studies of the effects of RI, compared with those of NF. This is particularly due to the different subdivisions/suborders of Oxisols, which have specific characteristics and can result in more consistent effects of RI vs NF on the common bean seed yield. It is worth noting that Oxisols are very weathered soils, with a small reserve of nutrients for plants, normally represented by their low to medium cation exchange capacity. In addition, the vast majority of Oxisols (more than 95%) are dystrophic and acidic, with pH between 4.0 and 5.5 and extremely low levels of available P, almost always below 1 mg dm^{-3} (Bockheim et al. 2014; Hartemink et al. 2020). These characteristics do not match the results that we will address below for the physicochemical attributes of the soil, which leads us to assume that, more important than the original soil class (inherent unfavorable quality), it is the proper management of this to improve its physicochemical characteristics, consequently enhancing the symbiosis between plants and bacteria.

Furthermore, our finding that seed yield is increased due to RI, especially under no-tillage cultivation conditions, is consistent with the notion that no-tillage is one of the most efficient ways to protect and improve the physicochemical characteristics of soil, in addition to being an alternative to relieve certain environmental tensions that may affect the symbiotic efficiency between plants and bacteria (Buffett 2012; Kaschuk et al. 2006; Omondi et al. 2014). In a study by Mulas et al. (2015) which was carried out under different environments and tillage systems, the BNF of common bean in no-tillage was higher than in the conventional planting system. Torabian et al. (2019) also reported in their study that no-till generally increases nodulation, the total amount of N fixation, and seed yield, compared with the conventional soil tillage system.

Other authors have confirmed this beneficial result of no-tillage, linked to RI, on the seed yield of the common bean (Giambalvo et al. 2012; Ruisi et al. 2012). These benefits go far beyond the agronomic part since it has also been reported that the association of the no-tillage system and RI promotes economic profitability and environmental benefits in the common bean cultivation system (Derpsch et al. 2010; Pittelkow et al. 2015; Soares et al., 2016). However, the effects of soil management, especially of no-tillage, on BNF vary depending on the time of implantation of the system and the edaphoclimatic conditions, which can present contradictory results for the seed yield of legumes (Torabian et al., 2019). It is worth mentioning that the benefits of the no-tillage system are particularly directed to regions with a tropical and temperate climate, as they help to maintain soil moisture, and consequently, favor BNF (Mulas et al., 2015).

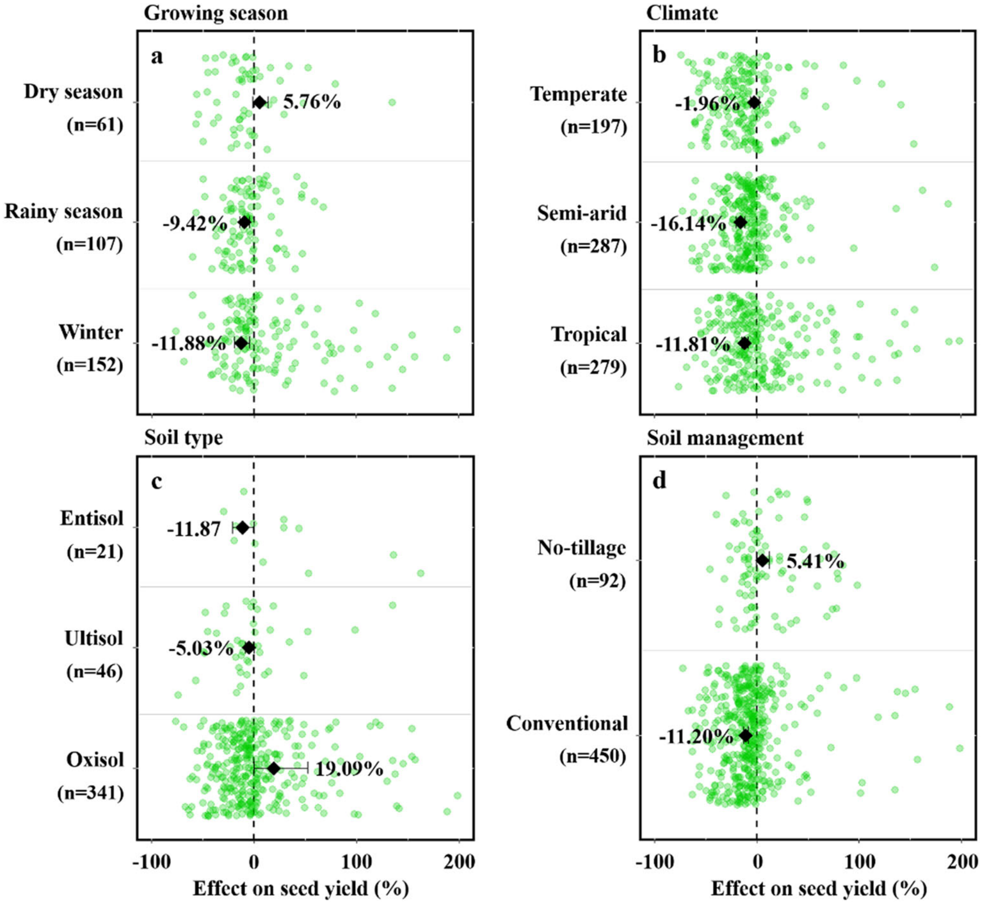

Fig. 6 Effects of *Rhizobium* inoculation compared with mineral-N fertilization (RI vs NF) on common bean seed yield as a function of growing season (**a**), climate (**b**), soil type (**c**), and soil management (**d**). The error bar represents the bootstrap confidence interval of 95% of the response rate. The green dots depict the observations of the sampled studies and the values in parentheses represent the sample size. The categories and their respective classes are described in Table 1.

3.3.2 Physical-chemical attributes of the soil

Overall, seed yield was lower for the RI treatment in most physicochemical attributes categorized in the present study (Figure 7). The lowest absolute values for seed yield under RI, compared to NF, were observed when using soil with clay content <60% (7.77%, although not significant), base saturation ≤ 50% (24 .64%), organic matter content ≤ 25 g dm^{-3} (18.66%), pH ≤ 5 and ≥ 7 (14.09% and 13.42%, respectively), and P availability ≤ 15 mg dm^{-3} (12.16%). Although the categories mentioned above did not show positive effects in general, we found that the RI performed better in soil with clay content ≥ 60%, base saturation ≥ 70%, soil pH between 5 and 7, and P availability > 15 mg dm^{-3}. This was an indication of a better performance of *Rhizobium*, attenuating the negative effect on seed yield, compared with NF.

In particular, an organic matter content of between 25 and 50 g dm^{-3} was the only factor categorized among the physicochemical attributes of the soil, which had a positive effect of RI on seed yield (2.48%), in comparison with that of NF. The positive effects of soil organic matter are well documented in the literature, which shows an increase in survival and the number of rhizobia in the soil, depending on the soil organic matter content, with an impact on both early nodulation and fixation efficiency of N_2 (Gopalakrishnan et al. 2015; Mohammadi et al. 2012). Adequate organic matter in the soil plays an important role in improving the physical, chemical, and biological properties of the soil and, consequently, improving or maintaining the sustainability of cultivation systems (Fageria 2012; Mikha and Rice 2004; Nyawade et al. 2019; Maitra et al. 2020), which is consistent with the positive results previously found for the no-tillage system. That is, these factors together have great potential to maximize BNF and reduce the use of chemical N fertilizers.

Many factors are related to the success of BNF in common beans through RI, mainly the type of regional climate where the common bean is cultivated. These factors are decisive for the reduction of BNF limitations, concomitantly improving the production of common beans (Al-Falih 2002). In addition to the environmental factors reported throughout this work, we highlight the typical characteristics of the soil, such as clay content, base saturation, soil pH, and P availability, which interfere with the symbiotic efficiency of *Rhizobium*, which is consistent with our meta-analysis (Adhikari et al. 2012; Argaw, 2016; Bambara and Ndakidemi 2010; Ju et al. 2019; Rego et al. 2015). Thus, our results corroborate previous evidence and indicate an ideal range of physicochemical attributes of the soil, which can be of great benefit to farmers who

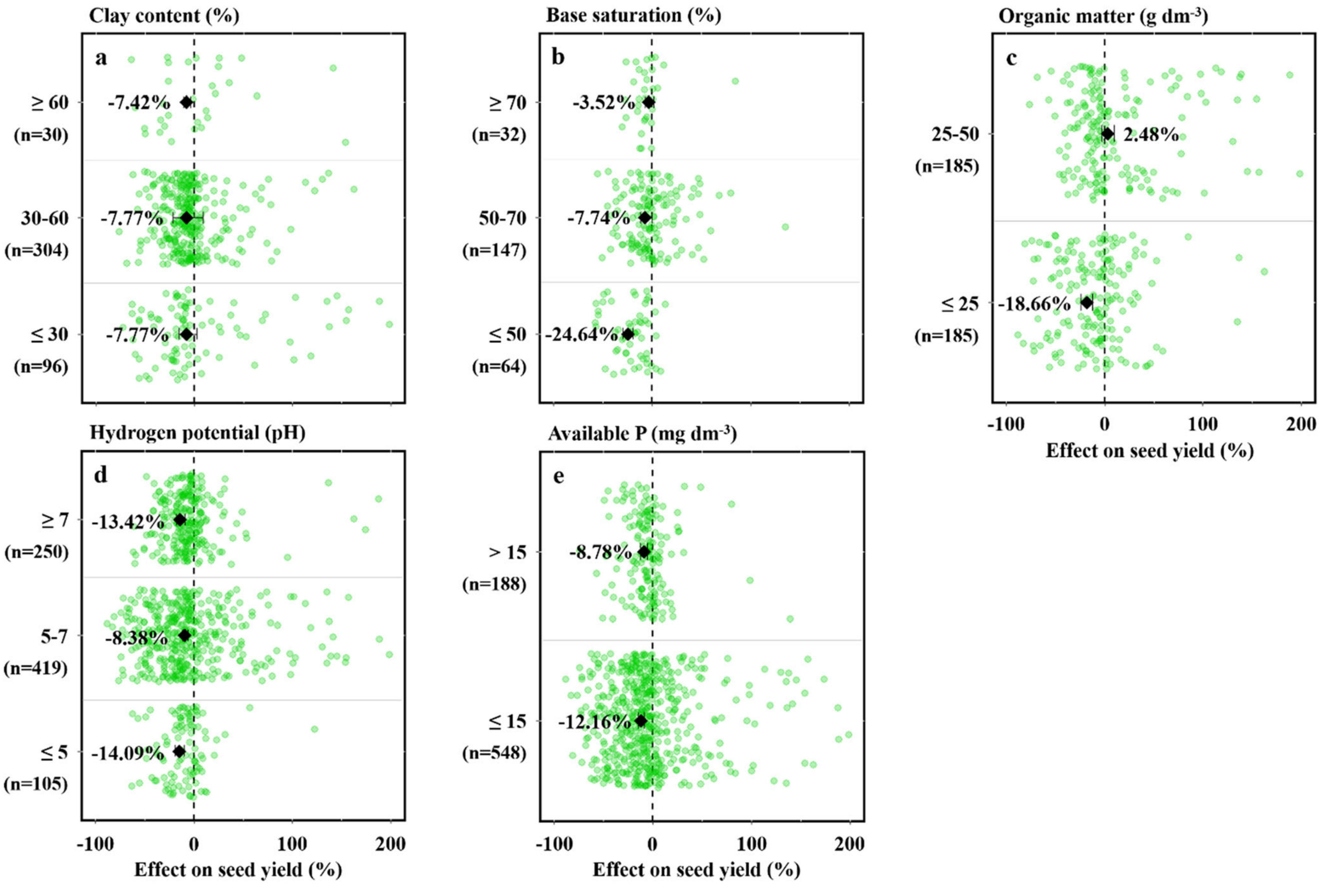

Fig. 7 Effects of *Rhizobium* inoculation compared with mineral-N fertilization (RI vs NF) on common bean seed yield as a function of clay content (**a**), base saturation (BS) (**b**), organic matter (OM) content (**c**), hydrogen potential (**d**), and availability of P in the soil (**e**). The error bar represents the bootstrap confidence interval of 95% of the response rate. The green dots depict the observations of the sampled studies and the values in parentheses represent the sample size. The categories and their respective classes are described in Table 1.

grow common beans using only RI under these specific soil conditions (Koskey et al. 2017; Makoi et al. 2013; Oliveira et al., 2017).

3.3.3 Characteristics of inoculation, phosphate fertilization, and nitrogen fertilization

The inoculant dose of 10 g kg^{-1} of seed negatively impacted the seed yield to a lesser degree (-4.06%) than that with the dose <10 or equal to 100 g kg^{-1} of seed, with the respective values of -21.69% and -12.20%, compared with that with NF (Figure 8a). The amount above a strain in the inoculant composition and the isolated type strain used in the inoculant further decreases seed yield to a greater degree compared with that with NF, with reductions of 14.56% and 13.36%, respectively. When using a type of strain in the composition of the inoculant and strains of the commercial type, the reduction in the seed yield was mitigated in comparison with that under NF, with reductions of 5.62% and 9.71%, respectively (Figure 8b and c).

It is important to mention the possible limitations of these results for the characteristics of the inoculant, as some studies have reported inaccurate classification of the inoculant used, such as not providing the concentration of viable cells, which should supply at least 1.2 million cells per seed, according to technical recommendations for common beans. In addition, inoculants must contain more efficient and competitive strains of *Rhizobium* (Hungria et al., 2003; Hungria et al., 2013) and, not necessarily under all conditions, commercial strains are more efficient than isolated strains, or that there is a formulation of the inoculant considered excellent for inoculating common beans. Thus, our results provide an overview of *Rhizobium* inoculation and should be carefully observed in this regard.

Inoculants based on the specificities of the region, taking advantage of adapted (commercial) strains as references and prioritizing the composition of the inoculant with only one strain, as this was the result that reduced seed yield to a lesser degree than that when more than one strain was used in the inoculant composition. According to Hassan et al. (2004), a mixture of strains will not always present results as successful as those of a single strain, suggesting that this mixture may cause an antagonistic effect, due to the production of bacteriocins, proteins, or protein complexes, with directed

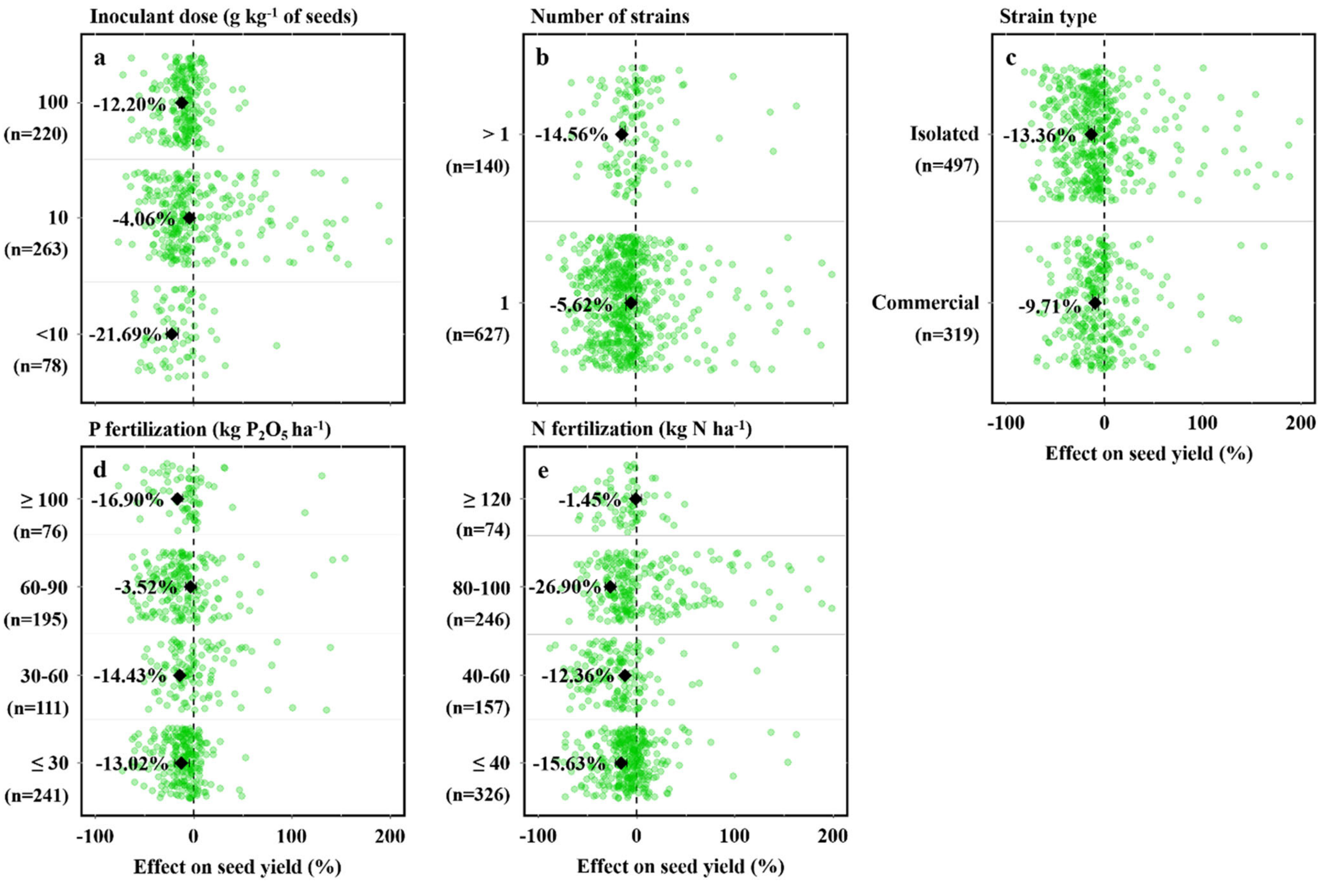

Fig. 8 Effects of *Rhizobium* inoculation compared with mineral-N fertilization (RI vs NF) on common bean seed yield as a function of doses of *Rhizobium* inoculant (RI) (**a**), the number of strains (**b**), strain type (**c**), P fertilization (**d**), and N fertilization (**e**). The error bar represents the bootstrap confidence interval of 95% of the response rate. The green dots depict the observations of the sampled studies and the values in parentheses represent the sample size. The categories and their respective classes are described in Table 1.

bactericidal activity (González et al. 2008; Hafeez et al. 2005). Thus, the results of the present study can serve as a basis for using local applications that aim to increase the efficiency of inoculation with rhizobia, developing an inoculant formulation suitable for local conditions, the so-called tailor-made inoculant (Mendoza-Suárez et al. 2020).

There was a lower seed yield, compared with that with NF, when RI was performed with rates of P fertilization ≥ 100 kg P_2O_5 ha^{-1} (16.90%) (Figure 8d). The negative results in seed yield promoted by RI were attenuated, especially at P rates between 60 and 90 kg P_2O_5 ha^{-1} (3.52). Deficiency and excess of P can reduce BNF by legumes (Demeterio et al. 1972; Schulze et al. 2006). Excessive P rates can induce zinc-deficient plants that nodulate less and are less productive (Demeterio et al. 1972). The effects of the combination of RI and P fertilization have previously been reported by other authors, demonstrating a potential effect on the seed yield of the common bean, as compared with that with NF (Kouki et al. 2016; Samago et al. 2018). Thus, an alternative that has been widely debated regarding P fertilization is to maximize the efficiency of P usage to benefit the symbiotic interactions in the plant's rhizosphere and consequently obtain a high crop yield, which can potentiate reductions in NF use (Bindraban et al. 2020).

For the categorization of NF rates, RI promoted a lower seed yield compared with that with NF (Figure 8e). The greatest reduction observed for RI regarding seed yield was obtained in comparison with that for the rates between 80 and 100 kg N ha^{-1} (26.90%). The effects on seed yield were attenuated when comparing RI with rates of NF ≤ 40 and between 40 and 60 kg N ha^{-1}, with reductions of 15.63% and 12.36%, respectively. We highlight the result obtained for RI compared with that with NF ≥ 120 kg N ha^{-1}, which resulted in the smallest reduction in seed yield (1.45%), which we consider a weak reduction to the point of not economically affecting the common bean seed yield. This was probably due to the negative effect of excessive N rates on common bean yield. According to Soratto et al. (2013), the demand for N by common beans ranges from 80 to 140 kg ha^{-1}. Mainly in the vegetative stages, excess of N may excessively increase leaf area, which may result in self-shading, consequently decreasing the photosynthetic efficiency and transpiration of common bean plants, and consequently reducing their seed yield (Soratto et al. 2014; Maia et al. 2017; Nasar et al. 2021).

Araujo et al. (2020) reinforce this discussion from an environmental point of view, stating that the environmental energy demand for the manufacture of an inoculant unit (defined as the inoculant needed for 1 ha), is less than 1% of that corresponding to the production of mineral-N fertilizer; that is, agricultural practices with RI constitute a more economical and sustainable environmental process than that for NF (Argaw and Muleta, 2018). However, our study revealed that, despite RI increasing the common bean seed yield, it does not have technical efficiency comparable to adequate rates of NF (Figures 5 and 8e).

The agronomic practices of mineral fertilization, regardless of P or N, should be further investigated considering the general economic aspects of the cultivation of common beans, as well as the cost/benefit ratio, variations in the cost of chemical fertilizers in countries that import a major part of these, mainly nitrogenous ones, and environmental costs affected by the adoption of NF (Hungria et al., 2013; Soares et al., 2016; Steiner et al., 2019). Added to this is the technological level at which the common bean producer is inserted because depending on the financial resources to which it has access, N inputs can become much more expensive than microbial inoculants (Hungria et al., 2013; Thilakarathna et al. 2019). Previous research adds to this discussion, stating that rhizobial inoculation will not always present higher yields; however, the technique contributes to reducing the rate of application of mineral N as well as that of economic and, consequently, environmental costs (Oliveira et al., 2017; Sousa et al., 2020). Overall, it can provide a better cost/benefit ratio than that of NF.

3.4 Recommendations for future research

In general, the results obtained in the present meta-analysis indicate the need for more experimental data, with a greater diversification of categorical factors, especially regarding the evaluated genus of N_2-fixing bacteria (*Rhizobium*). We highlight the few studies that tried to investigate the efficiency of *Rhizobium* strains/species on common bean seed yield, compared with NF. Thus, it is necessary to categorize the effects based on the different species of rhizobia that nodulate common beans, such as *R. leguminosarum* bv. *phaseoli*, *R. tropici*, *R. etli*, *R. gallicum*, and *R. giardinii*. It is also important to focus on other forms of inoculation, in addition to those done exclusively on the seed, such as inoculation in the furrow, topdressing application of inoculation, and co-inoculation. In future studies, these factors must be considered to produce more robust analyses, which will allow a more in-depth assessment of the real effect of RI on common beans.

Furthermore, it is still unclear for the common bean crop whether NF can be replaced entirely by RI, as there are still many dependencies on abiotic factors to achieve success with this inoculation technique, in addition to intrinsic factors of the genotype of common beans and the strains used in the inoculant. Therefore, we recommend that in future studies whose objective is to help producers to reduce N inputs, they should seek to relate rhizobial inoculations with rates of below 60 kg ha^{-1} of mineral N, as well as to associate the effects of RI with P fertilization. It is also important to consider all the categorized factors assessed in this meta-analysis and the local conditions in which common bean cultivation is being carried out.

It is important to highlight that most of the studies were conducted in tropical conditions, as in Brazil. Thus, the geographic variations of the globe, particularly in each location where the studies were conducted, must be taken into account in the present meta-analysis, as these may have contributed to any bias in the data analysis.

4 Conclusion

By studying the efficiency of inoculation with *Rhizobium* in the common beans it was possible to reveal that it consistently increases nodulation of plants, yield components, and seed yield. However, compared with mineral-N fertilization, the rhizobial inoculation does not have the same efficiency in increasing the number of pods per plant and seed yield of common beans, with the differences in seed yield varying in accordance with certain conditions (categories). All the factors studied in the present meta-analysis influenced the response of common bean seed yield as a function of inoculation with *Rhizobium*. Compared with NF, rhizobial inoculation was more effective in increasing seed yield when common beans were cultivated in the dry season, under a no-tillage system, and in soils with high organic matter content. This conclusion is one of the main contributions of our analysis, given the discrepancies about this process in the literature. In addition, a reduction of NF can be encouraged by more intense use of inoculation with *Rhizobium* (>10 g of rhizobial inoculum per kg of seeds), especially for the following soil characteristics: soils with a clay texture; eutrophics (base saturation > 50%); that are neutral to slightly acidic; and with adequate P availability.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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