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Meta-analysis of maize responses to *Azospirillum brasilense* inoculation in Brazil: Benefits and lessons to improve inoculation efficiency

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ABSTRACT

The inoculation of maize with the plant-growth-promoting bacteria (PGPB) *Azospirillum brasilense* Ab-V5 and Ab-V6 strains has impressively increased in Brazil in the last decade. In the present study, we conducted a meta-analysis with 60 studies published between 2010 and 2021, comprising 103 field trials in 54 locations in Brazil, aiming to assess the benefits and factors that affect the efficiency of *A. brasilense* inoculation of maize. Results showed that bacteria inoculation increased 12.1% of root mass, 4.3% N leaf concentration, 5.4% grain yield, and 3.6% of N in grains. The analysis of cultivars, edaphoclimatic conditions, and others detected positive effects of inoculation on maize under all the study conditions and yield ranges. However, inoculation benefits were higher at yields ≤3000 kg/ha (+21%) than between 3000 and 12,000 kg/ha (+1.5% to +6.2%), and yield responses tend towards greater increases at lower N rates (≤ 50 kg/ha, +8%) that at higher ones (> 200 kg/ha, +3.8%). Seed inoculation was more efficient than inoculation via leaf spray, especially by applying solid (peat) inoculants (+9.5%) than liquid formulations (+5.5%). Leaf-spray inoculation showed positive effect on grain yield (+3.1%) only when performed at the initial vegetative growth stages (V2–V3). *A. brasilense* inoculation represents an important biotechnology tool to increase yields and nutritional value of maize crops under most agronomic and environmental tropical and subtropical conditions.

1. Introduction

Plant growth-promoting bacteria (PGPB) can benefit crop production by a variety of single or combined mechanisms. The most cited effects refer to biological nitrogen fixation (BNF), synthesis of phytohormones, phosphorus solubilization, and induction of systemic resistance to abiotic and biotic stresses (Bashan and Holguin, 1998; Fukami et al., 2017; Olanrewaju et al., 2017; Swarnalakshmi et al., 2020). After rhizobia, the most worldwide studied and applied PGPB as inoculants belong to the genus *Azospirillum* (Döbereiner and Pedrosa, 1987; Okon and Labandera-Gonzalez, 1994; Bashan and de-Bashan, 2010; Fukami et al., 2018a; Pereg et al., 2016; Cassán et al., 2020; Santos et al., 2019, 2021a). *Azospirillum* spp. mechanisms to promote plant growth include BNF, root development by the synthesis of phytohormones, and enhancement of membrane activity. These mechanisms lead to higher nutrient and water uptake, mitigation of abiotic stress, such as salinity and drought, and induction of systemic resistance to pathogens (Bashan and de-Bashan, 2010; Hungria et al., 2010; Fukami et al., 2017; Cassán et al., 2020; Santos et al., 2021a, 2021b). Hundreds of studies performed in a number of different countries have reported benefits of *Azospirillum* spp. inoculation (Okon and Labandera-Gonzalez, 1994; Baldani and Baldani, 2005; Barbosa et al., 2012; Cassán et al., 2020; Santos et al., 2021a). Interestingly, a review by Pereg et al. (2016) showed that *Azospirillum* spp. promoted the growth of 113 plant species across 35 botanical families, including 14 species of cereals, which indicates that the genus encompasses strains effective to practically every tested plant species so far.

Johanna Döbereirner set up the first studies on nitrogen-fixing *Azospirillum* spp. in Brazil (Baldani and Baldani, 2005), and, she isolated the bacteria and drawn strategies to employ the concept of nitrogen fixation in crop production after years of efforts. She confirmed that *Spirillum lipoferum* was the main nitrogen-fixing bacterium associated with roots

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of the forage grass *Digitaria decumbens* (Stent) (Döbereiner and Day, 1976). The ability of *S. lipoferum* to fix nitrogen when associated with grasses was soon confirmed, leading to the reclassification of the genus as *Azospirillum* (Tarrand et al., 1978). Several other reports have followed in many countries, including studies on taxonomy, ecology, plant-microbe interactions, quantification of BNF, synthesis of phytohormones, among others (Döbereiner and Pedrosa, 1987; Bashan and de-Bashan, 2010; Cassán et al., 2020).

Despite the Brazilian leadership in studies on *Azospirillum* spp., there were no inoculants available in the country until 2009, when *A. brasilense* Ab-V5 (=CNPSo 2083) and Ab-V6 (=CNPSo 2084) strains were launched in the market. They were identified during a selection program for maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) inoculation (Hungria et al., 2010). There was a high interest in studies of such strains in the country (Santos et al., 2021a, 2021b), including the expansion to other crops such as rice (*Oryza sativa* L.), *Urochloa* spp. pastures, co-inoculation of soybean (*Glycine* max L.) Merr.), and common bean (*Phaseolus vulgaris* L.) (Hungria et al., 2013, 2016; Ferreira et al., 2020a; Barbosa et al., 2021; Santos et al., 2021a). The inoculants have also gained prominence among Brazilian farmers, reaching the annual commercialization of 10.5 million doses in 2020 (Santos et al., 2021a).

Nowadays, maize is cultivated in 19.09 million hectares in Brazil (Mha) and is the most inoculated cereal in the country with *A. brasilense* Ab-V5 and Ab-V6 strains. Inoculation trials had initially set to supply a starter dose of 24 kg/ha of N and they reached yields of 4000 kg/ha (Hungria et al., 2010). In addition to N starter, supplying another 45 kg/ha of N as cover fertilization resulted in yields of 6000 kg/ha, increasing to 8000 kg/ha after the application of 67,5 kg/ha of N as cover fertilization as well (Hungria, 2011; Hungria and Nogueira, 2019). Despite these promising results, the assumptions of maize responses to *A. brasilense* inoculation have been based on a very limited number of field trials.

Meta-analysis has proved to be a powerful tool for the advance of scientific knowledge towards agronomic advice (Barbosa et al., 2021). Here we report a meta-analysis based on results of fields trials on maize crops inoculated with *A. brasilense* in Brazil. Results pointed to interesting conclusions and useful information to guide farmers for the use of inoculants in order to increase the sustainability of crop production. We hypothesized that inoculation of maize with *A. brasilense* has positive effects on crop development under the most varied agronomic and environmental Brazilian conditions.

2. Material and methods

2.1. Data sources and treatments

A systematic survey was carried out on the Web of Science and Google Scholar platforms between October 2020 and February 2021 based on the following keyword combinations: "maize" or "corn" or "*Zea mays*" and "inoculation" or "*Azospirillum brasilense*" and "Brazil" or "Brazilian". Publications were reviewed to check for the following criteria: (1) article published in peer-reviewed scientific journal; (2) study conducted under field conditions in Brazil; (3) treatments without inoculation (control treatment) and with inoculation of *A. brasilense* Ab-V5 and/or Ab-V6 strains (treatment); (4) availability of results that could be directly extracted from the text, tables and/or figures. It is important to highlight that the choice of these *A. brasilense* strains was because of their wide application in Brazil and to reduce interferences in the meta-analysis, which may occur when several strains are selected.

After a careful evaluation, 60 publications were selected for the meta-analysis as follow: Hungria et al., 2010; Godoy et al., 2011; Ferreira et al., 2013; Kappes et al., 2013; Martins et al., 2012; Araújo et al., 2014a; Araújo et al., 2014b; Cunha et al., 2014; Mazzuchelli et al., 2014; Nakao et al., 2014; Costa et al., 2015; Marks et al., 2015; Matsumura et al., 2015; Pandolfo et al., 2015; Piccinin et al., 2015; Sangoi et al., 2015; Santos et al., 2015; Silva et al., 2015; Andrade et al., 2016; Brum et al., 2016; Cadore et al., 2016; Fukami et al., 2016; Galindo et al., 2016; Kaneko et al., 2016); Longhini et al., 2016; Morais et al., 2016; Martins et al., 2016; Milléo and Cristófoli, 2016; Müller et al., 2016; Spolaor et al., 2016; Garcia et al., 2017; Guimarães et al., 2017; Matos et al., 2017; Garbin and Simonetti, 2017; Oliveira et al., 2017; Quintão et al., 2017; Szilagyi-Zecchin et al., 2017; Aosani et al., 2018; Galindo et al., 2018; Moreira et al., 2018; Oliveira et al., 2018; Souza et al., 2018; Alvarez et al., 2019; Modesto et al., 2019; Pereira et al., 2019; Zambonin et al., 2019; Gavilanes et al., 2020; Alves et al., 2020; Bassetto Júnior et al., 2020; Carmo et al., 2020; Coelho et al., 2020; Fernandes et al., 2020; Ferreira et al., 2020b; Galindo et al., 2020; Marques et al., 2020; Machado et al., 2020; Pedrosa et al., 2020; Pereira et al., 2020; Rocha et al., 2020; Caires et al., 2021; Müller et al., 2021). The selected publications referred altogether to 103 field trials conducted in 54 locations in Brazil (Fig. 1).

Data were extracted from (1) data from the first publication, when different publications used the same dataset; (2) each year, which was compared to other years when the data were provided separately over the years; (3) replicates for each year, which were multiplied by the number of years when values expressed averages over the years. General information from each study was initially obtained, such as location, soil texture, soil organic matter content, maize cultivar, soil management system, inoculant type, species, and method of inoculation with *A. brasilense*. Mean values (X), standard deviations (SD), and number of replicates (n) were obtained from publications, which evaluated grain yield, root mass, and N concentration in leaves and grains of maize plants. Eq. (1) was applied to obtain SD values from publications that only reported the coefficient of variation (CV%):

$$SD = \frac{CV\%}{100} X \tag{1}$$

An average value of CV% for the control and treatments was calculated from SD values (Eq. (2)) for those publications that showed no information on data variability. Then, the obtained values were applied in Eq. (1).

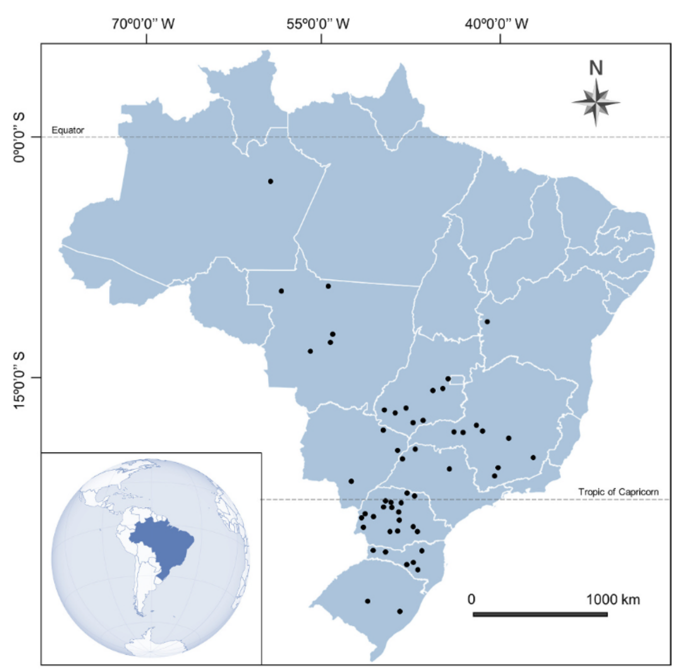

Fig. 1. Experiment locations in the 60 publications used in the meta-analysis, which correspond to 54 locations in 10 Brazilian states where 103 field trials were carried out.

$$CV\% = \frac{SD}{X}100 \quad (2)$$

All data were extracted and compiled in an Excel® spreadsheet.

2.2. Controlling factors

The following groups of controlling factors were considered to evaluate maize responses to inoculation with *A. brasilense*: method of inoculation, grain yield, N fertilization, cultivar characteristics, and edaphoclimatic factors (soil texture, soil organic matter content, management system, and climate type). Only groups that presented at least 15 paired comparisons (control x treatment) were used in the analysis.

For inoculation of *A. brasilense* we considered application in seeds and leaves. The inoculation has usually applied two strains simultaneously (Ab-V5 and Ab-V6) or only with Ab-V5, by using either liquid or solid inoculants (peat). For leaf spray, the inoculation of *A. brasilense* considered maize phenological stages V2 to V6, and spray volume from 50 to 300 L/ha. It is worth mentioning that some studies performed inoculation either in sowing furrow or on the soil surface. However, due to the low number of observations, it was not possible to analyze these inoculation methods as controlling factors, and such data were only used for the global meta-analysis.

Five yield ranges were considered in the meta-analysis: ≤3000 kg/ha; 3001–6000 kg/ha; 6001–9000 kg/ha; 9001–12,000 kg/ha; >12,000 kg/ha. Total N rates applied to maize crops considered the sum of application rates at sowing and topdressing. Based on the variation verified in the studies, it was possible to organize the following N fertilization ranges: ≤50 kg/ha; 51–100 kg/ha; 101–150 kg/ha; 151–200 kg/ha; >200 kg/ha.

The characteristics of maize cultivars were categorized according to the hybrid crossing (single, double, and triple) and cycle type (very early and early). The effect of maize genetic modification was categorized as conventional and also according to the event of genetic modification: TC1507 x MON810 – confers resistance to the glufosinate herbicide and lepidopterans (introduced genes cry1Fa2, pat and cry1Ab); MON89034 x TC1507 x NK603 – confers resistance to the glufosinate and glyphosate herbicides and lepidopterans [introduced genes cry1Fa2, pat, cry1A.105, cp4 epsps (aroA:CP4) and cry2Ab2]; MON89034 x NK603 – confers resistance to the gliphosate herbicide and lepidopterans [introduced genes cry1A.105, cp4 epsps (aroA:CP4) and cry2Ab2]; MON89034 – confers resistance to the lepidopterans (introduced genes cry1A.105 and cry2Ab2); TC1507 – confers resistance to the glufosinate herbicide and lepidopterans (introduced genes cry1Fa2 and pat). The information on cultivars was obtained from each publication and from disclosure texts of companies that registered a specific cultivar, when necessary. Yet, information on genetic modifications of maize cultivars was obtained from ISAAA (2021).

The edaphoclimatic factors were categorized in tropical and subtropical climates, conventional tillage and non-tillage, soil texture (sandy, loam, and clayey), and organic matter contents (OM) (>4%, 4–2.1% and ≤ 2%).

2.3. Meta-analysis

The magnitude of the inoculation effect on maize was calculated using the natural logarithm of the response ratio (lnRR; Eq. (3)) as effect size (Hedges et al., 1999):

$$\ln RR = ln\frac{X_e}{X_c} \quad (3)$$

where X_e e X_c are the mean values for respectively treatments and control. The variance (v) was calculated according to Eq. (4):

$$v = \frac{SD_e^2}{n_e X_e^2} + \frac{SD_c^2}{n_c X_c^2} \quad (4)$$

where SD_e, n_e, SD_c and n_c represent the standard deviation and the number of replicates for respectively treatments and control. Inoculant effects on maize were considered significant when the 95% of confidence interval (CI) values for response ratio did not overlap with zero. The average values of RR and CI were generated using the random-effects method with restricted maximum likelihood estimation. To facilitate the interpretation of variations between treatments and control, the RR and the CI values were transformed into percentage (Eq. (5)):

$$\%change = \left(e^{\ln RR} - 1\right) \times 100 \quad (5)$$

The robustness of the meta-analysis was assessed by fail-safe N according to the method proposed by Rosenberg. Fail-safe N indicates the number of studies (unpublished or absent) of null effect that should be added to a meta-analysis to change the results from significant to non-significant (Rosenberg, 2005). Results of meta-analysis have been considered robust for fail-safe N values greater than 5n + 10 (Rosenthal, 1991). All analyzes were performed in the OpenMEE software (Wallace et al., 2017) and the figures were drawn in the SigmaPlot software.

3. Results

The majority of the global average data (all data) and groups of controlling factors presented satisfactory results in the fail-safe N test, being higher than the values of 5n + 10 (Table 1). However, there were four exceptions for fail-safe N values lower than 5n + 10 among the 43 conditions analyzed: global average data - roots mass; groups – grain yield greater than 12,000 kg/ha, triple cross hybrids, and clayey soils.

A. brasilense inoculation caused significant effects on grain yield (kg/ha), root mass (g/plant), concentration (%) of N in leaves and in grains (Fig. 2). The greatest benefit of inoculation was the development of root system (+12.1% root mass), followed by increments of 5.4% in grain yield, and in concentrations of N in leaves (4.3%) and grains (3.6%).

Grain yields positively responded to seed inoculation with both peat (+9.5%) and liquid inoculants (+5.5%), but there was no significant effect of leaf spray inoculation when all data were analyzed together. Leaf spray inoculation significantly affected maize yield between vegetative development stages V2 and V3 (+3.1%), and grain yield increased by 8.6% and 5.1% following the inoculation with either Ab-V5 or Ab-V5 and Ab-V6, respectively (Fig. 3).

The inoculation had a positive effect on grain yield in all yield ranges and N rates (Fig. 4). There was a greater response of yields ≤3000 kg/ha (+21%) to inoculation compared to the other yield ranges (+1.5% to +6.2%). There was also a trend of decrease in response to inoculation with increasing N application rates to soils, especially at rates ≤50 kg/ha (+8%) and > 200 kg/ha (+3.8%).

Inoculation increased grain yield for the events of maize genetic modification in all controlling genetic factors (Fig. 5). The largest increases in grain yield occurred for the events of genetic modification TC1507 x MON810 (+9.2%) and MON89034 x TC1507 x NK603 (+7%), compared to the MON89034 (+3%) and TC1507 (+2.2%), as there was no overlap between the confidence intervals of these events.

The inoculation of maize was beneficial for grain yield when the analysis considered soil organic matter content (+5.9% to +3.6%), soil texture (+8.3% to +5.3%), and tillage management system (+4.6% to +3.2%) (Fig. 6). Results based on each climate type also indicated beneficial effects on grain yield, but the response intensity was higher in subtropical (+7%) than in tropical (+2.6%) climate.

4. Discussion

As previously mentioned, the *A. brasilense* Ab-V5 (= CNPSo 2083) and Ab-V6 (= CNPSo 2084) strains resulted from a Brazilian selection program for maize and wheat crops (Hungria et al., 2010; Hungria and Nogueira, 2019). Brazilian legislation demands that inoculants must

Table 1
Results of Rosenberg's fail-safe N test for the data used in the meta-analysis of maize response to inoculation with *Azospirillum brasilense* strains Ab-V5 and Ab-V6 in Brazil.

Data groups	Plant attributes	Samples number (n)	*Fail-safe* N	5n + 10
All data	Grain yield	493	91,906	2475
All data	N in grains	89	2993	455
All data	N in leaves	139	1321	705
All data	Root mass	19	88	100
Inoculation method and *A. brasilense* strains				
Ab-V5 + Ab-V6	Grain yield	386	52,528	1940
Ab-V5	Grain yield	69	3441	355
Seed/peat	Grain yield	45	363	235
Seed/liquid	Grain yield	325	39,605	1635
Leaf spay/liquid	Grain yield	85	924	435
Vegetative stage of maize at the leaf spray inoculation				
V2 to V3	Grain yield	44	700	230
V4 to V6	Grain yield	34	211	180
Grain yield class (kg/ha)				
>12,000	Grain yield	25	72	135
9001–12,000	Grain yield	130	4074	660
6001–9000	Grain yield	228	29,092	1150
3001–6000	Grain yield	91	739	465
≤3000	Grain yield	38	2860	200
N fertilization (kg/ha)				
>200	Grain yield	25	512	135
151–200	Grain yield	66	2516	340
101–150	Grain yield	107	2745	545
51–100	Grain yield	111	4124	565
≤5	Grain yield	96	4864	490
Hybrid crossing and cycle				
Simple	Grain yield	322	5175	1620
Double	Grain yield	32	581	170
Triple	Grain yield	55	60	285
Very Early	Grain yield	78	3111	400
Early	Grain yield	346	23,862	1740
Maize genetic modification				
No	Grain yield	165	12,464	835
Yes	Grain yield	282	16,096	1420
Event of maize genetic modification				
TC1507 x MON810	Grain yield	20	111	110
MON89034 x TC1507 x NK603	Grain yield	49	2049	255
MON89034 x NK6	Grain yield	23	313	125
MON89034	Grain yield	34	269	180
TC1507	Grain yield	94	952	480
Environmental factors (soil texture, organic matter (OM) and management system; climate)				
Sandy	Grain yield	30	178	160
Loam	Grain yield	111	5882	565
Clayey	Grain yield	324	324	1630
OM > 4%	Grain yield	61	1102	315
OM 4–2.1%	Grain yield	248	24,497	1250
OM ≤ 2%	Grain yield	101	2333	515
Non-tillage	Grain yield	237	21,353	1195
Conventional tillage	Grain yield	70	964	360
Subtropical	Grain yield	310	56,174	1560
Tropical	Grain yield	179	4423	905

contain strains exclusively generated from specific research protocols (MAPA, 2011). The strains Ab-V5 (= CNPSo 2083) and Ab-V6 (= CNPSo 2084) were approved as inoculants in 2009 and have been released without restrictions for studies by both the public and private sectors in Brazil and in other countries (Santos et al., 2021a). Since then, seed inoculation and leaf-spray with Ab-V5 and Ab-V6 strains (Fukami et al., 2016; Hungria et al., 2021) have extended to other grasses such as *Urochloa* spp. (Hungria et al., 2016, 2021). The co-inoculation of legumes (Hungria et al., 2013) has also rendered benefits confirmed by farmers, who have applied 10.5 million doses of inoculants containing

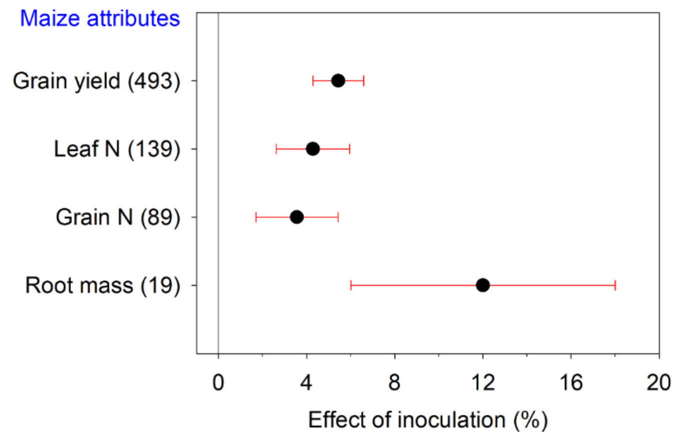

Fig. 2. Effect (%) of inoculation of maize with *Azospirillum brasilense* on grain yield, N concentrations in leaves and grains, and root mass. Values are means ±95% of the confidence interval (CI) for inoculation effects. Number of comparisons for each maize attribute is in parentheses. The effect is significant when the CI does not overlap the zero.

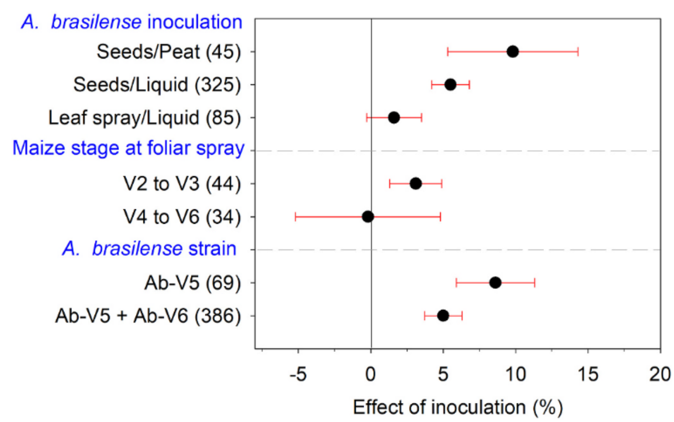

Fig. 3. Effect of inoculation with *Azospirillum brasilense* on maize grain yield according to the inoculation method and *A. brasilense* strain. Values are means ±95% of the confidence interval (CI). Number of comparisons for each maize attribute is in parentheses. The effect is significant when the CI does not overlap the zero.

the strains Ab-V5 and Ab-V6 in the 2019/2020 crop season (Santos et al., 2021a). The number of studies on these two strains in all Brazilian biomes has currently highlighted their importance in the country (Santos et al., 2021a). Therefore, it is important to analyze metadata to verify the consistency of inoculation responses and key points that should be better studied to improve recommendations to farmers. Another meta-analysis study on soybean for instance has confirmed the benefits of co-inoculation with *A. brasilense* strains in comparison to inoculation exclusively with *Bradyrhizobium* spp. (Barbosa et al., 2021).

Now, in our current meta-analysis it was confirmed the agronomic efficiency of inoculation of maize with *A. brasilense* Ab-V5 and Ab-V6 strains (Fig. 2), improving grain yield by an average of 5.4%. Díaz-Zorita et al. (2015) have also reported positive effects of inoculation of *A. brasilense* (isolated Az39) in 81.1% of their study, which analyzed data from 316 field experiments in Argentina. There have also been studies indicating high economic viability of *A. brasilense* Ab-V5 and Ab-V6 inoculation in maize (Galindo et al., 2018; Caires et al., 2021) and in other crops (Bárbaro-Torneli et al., 2017; Ferreira et al., 2020a; Prando et al., 2020). Thus, maize inoculation provides economic and environmental gains, both important for the sustainable intensification of productive systems.

Root system of maize was the plant attribute that has showed the

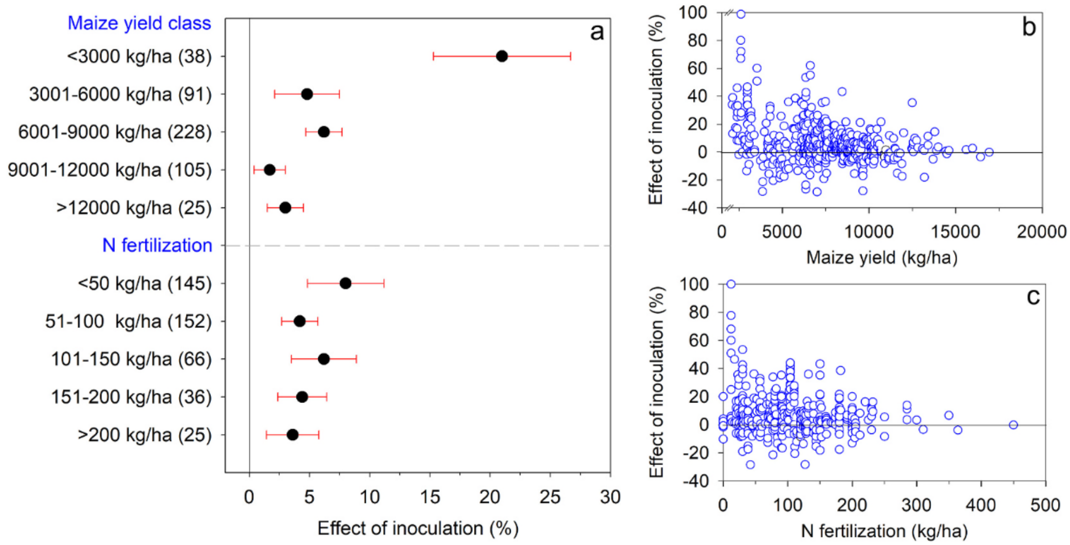

Fig. 4. Effect of *Azospirillum brasilense* inoculation on maize grain yield according to the yield range and N fertilization rates (a), and data dispersion according to each yield (b) and N rates (c). Values are means ±95% of the confidence interval (CI). Number of comparisons for each maize attribute is in parentheses. The effect is significant when the CI does not overlap the zero.

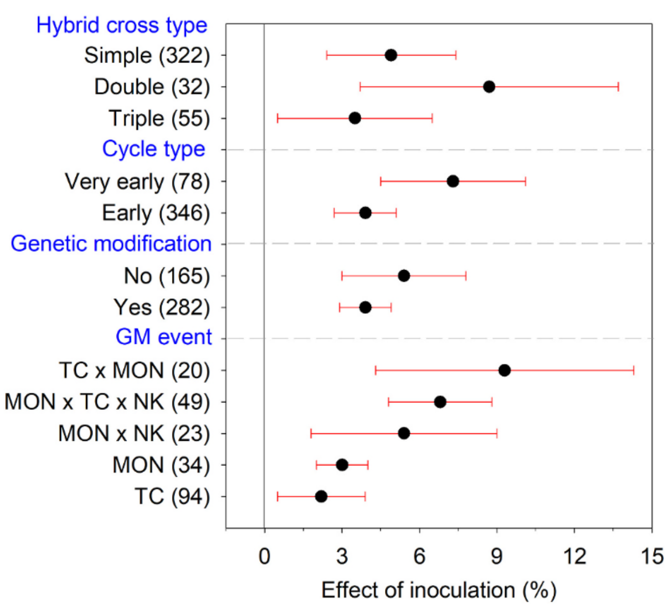

Fig. 5. Effect of *Azospirillum brasilense* inoculation on maize grain yield according to hybrid, cycle, and genetic modification of cultivars. Values are means ±95% of the confidence interval (CI). Number of comparisons for each maize attribute is in parentheses. The effect is significant when the CI does not overlap the zero. Genetically modified maize: TC x MON – event TC1507 x MON810; MON x TC x NK – event MON89034 x TC1507 x NK603; MON x NK – event MON89034 x NK603; MON – event MON89034; TC – event TC1507.

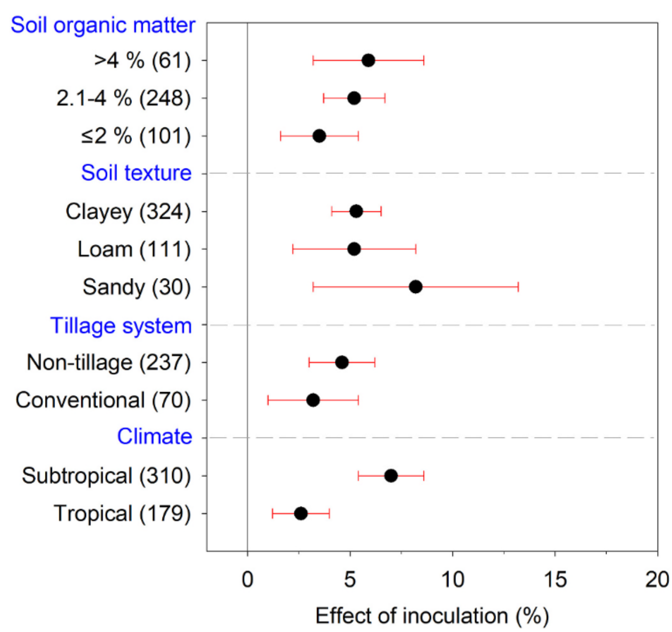

Fig. 6. Effect of *Azospirillum brasilense* inoculation on maize grain yield according to soil properties (texture, organic matter content, soil tillage management; and climate). Values are means ±95% of the confidence interval (CI). Number of comparisons for each maize attribute is in parentheses. The effect is significant when the CI does not overlap the zero.

highest response to inoculation, which was probably the main factor responsible for the benefits verified in shoots and grains (Fig. 2). Several studies have shown that inoculation with *A. brasilense* provides significant increases in root growth, root hairs, and other root parameters (Garcia et al., 2017; Garbin and Simonetti, 2017; Galindo et al., 2020; Rondina et al., 2020; Santos et al., 2020b; Hungria et al., 2021). It is known that one of the greatest benefits of *A. brasilense* is the synthesis of several phytohormones and plant regulators (Cassán et al., 2020), and the strains Ab-V5 and Ab-V6 synthesize indole-acetic acid (AIA), indole-3-ethanol, and salicylic acid; they also synthesize jasmonic acid, gibberellic acid (GA_3), and indole-3-lactic acid in lower concentrations (Fukami et al., 2017; Fukami et al., 2018b). As a result of an increased root system, the acquisition of water and nutrients is optimized. Improvements in water acquisition by plants inoculated with Ab-V5 and Ab-V6 strains have been reported in soybeans (Cerezini et al., 2016), and *Urochloa* spp. (Leite et al., 2019), while increasing accumulation of N in plant tissues has been reported for maize (Hungria et al., 2010; Martins et al., 2018), *Urochloa* spp. (Hungria et al., 2016, 2021), and co-inoculated soybean (Barbosa et al., 2021), including increases in the efficiency of use of N from fertilizers (Martins et al., 2018). In addition, the increase in the rhizodeposition of organic molecules can directly affect the acquisition of water and nutrients, which can indirectly favor the development of microorganisms that promote plant growth in the rhizospheric environment (Jones et al., 2009; Barbosa et al., 2015). However, the number of root data used in this meta-analysis was relatively low (Fig. 2). It would be interesting that new studies focus on responses of roots to *A. brasilense* inoculation.

Special emphasis should be given to the increases of N concentrations in leaves and grains (Fig. 2), which occur via biological nitrogen fixation (BNF) and higher absorption of nutrients due to greater root development. Studies using ^{15}N isotope have contributed to understand the importance of different N sources in inoculated maize plants. Salamone et al. (1996) have reported that BNF contributed with 48% to 58% of the N accumulated in maize plants inoculated with *A. brasilense* (isolated 42 M), while Araújo et al. (2015) have reported increases of 19% after maize inoculation with strains Ab-V5 and Ab-V6. Martins et al. (2018) have found that maize utilization of N from urea was 58% of the amount applied to plants inoculated with *A. brasilense* (Ab-V5 and Ab-V6 strains) against 34% for plants that were not inoculated (control). Therefore, both BNF and increased uptake of N-fertilizer should contribute to the benefits observed after maize inoculation with *A. brasilense*.

The concentration of nutrients in maize grains is an attribute of high importance because of the nutritional value of grains for humans and livestock, and the inoculation of maize was efficient in improving this attribute (Fig. 2). Such an increase is probably related to the higher N concentration in leaves (Fig. 2), which together with the stalks are often the main sources of N for grains (Ning et al., 2017; Ray et al., 2020). The increase in the N concentration in leaves (Fig. 2) has also beneficial effects on animal nutrition due to the use of the entire aerial part of maize for silage production. Additionally, there is evidence that the benefits of inoculation are not restricted to N as there are reports on increments of P, K, Mg, S, Zn, Mn, and Cu in maize (Hungria et al., 2010) and K in *Urochloa* spp. (Hungria et al., 2021) following the inoculation with the strains Ab-V5 and Ab-V6.

4.1. *Inoculant carriers and methods of inoculation*

Seed is the most common way of *A. brasilense* inoculation of maize, especially with liquid inoculants, which represent the preferred carriers and respectively accounts for 80% and 96% of the market in Brazil and in other South American countries, (Santos et al., 2019; Cassán et al., 2020). Solid inoculants (peat) are less used, but are considered as “gold standards” due to their physicochemical properties that provide protection and nutrients to the bacteria (Hungria et al., 2005). In this meta-analysis, peat inoculants showed similar or slightly higher efficiency compared to liquid inoculants when applied to maize seeds (Fig. 3). Seed inoculation can stand out because bacteria colonize the rhizosphere and root tissues more efficiently (Marks et al., 2015; Fukami et al., 2016; Coelho et al., 2020).

Alternative methods of inoculation have been proposed, including in-furrow at sowing, soil surface spray and leaf spray (Fukami et al., 2016; Oliveira et al., 2017; Machado et al., 2020). Alternative methods of inoculation deal mainly with limitations imposed by the seed treatment with pesticides incompatible with *A. brasilense* (Santos et al., 2020a, 2020b, 2021b). In our meta-analysis it was possible to verify that the efficiency of leaf spray inoculation has varied with the vegetative development stage of maize, with a positive effect only when the inoculation was carried out between stages V2 and V3 (Fig. 3). Under such condition, leaf spray inoculation has provided similar benefits as seed inoculation with liquid inoculants. Leaf-spray inoculation in early vegetative stages of maize may be more efficient because the bacteria will interact with the plant for a longer time. In addition, part of the spray solution reaches the soil, where rhizospheric and root tissues may also receive the bacteria. However, it is important to highlight that there have been benefits of leaf-spray inoculation of maize with *A. brasilense* Ab-V5 and Ab-V6 even under controlled conditions when bacteria were prevented to reach the soil with residues of inoculation solution (Fukami et al., 2016), suggesting other beneficial effects such as induction of systemic tolerance to abiotic stresses (Fukami et al., 2018a). As any biological product inoculated in seeds, leaf spray inoculation needs to be carried out under conditions that do not harm the survival of bacteria such as extreme air temperatures and solar radiation (Jones et al., 2012; Preininger et al., 2018). However, the number of studies on alternative methods of inoculation is still small and there is the need of more comparative trials to draw conclusions on the subject, especially with leaf-spray and in-furrow inoculation.

4.2. *Grain yield*

The highest responses of maize to inoculation have occurred for grain yields ≤3000 kg/ha (Fig. 4a,b). As already discussed, inoculation brings benefits that give maize plants greater efficiency in the use of water and nutrients (Bashan and de-Bashan, 2010; Hungria et al., 2010; Fukami et al., 2017; Cassán et al., 2020; Santos et al., 2021a, 2021b), which are essential resources that can severely limit yield potential of maize (Subedi and Ma, 2009; Barbosa et al., 2016). Consequently, we hypothesize that there has been greater limitation of essential resources at grain yields ≤3000 kg/ha that boosted the effects of inoculation compared to other grain yield ranges. In spite of it, the inoculation has brought gains of grain yields in all the evaluated ranges (Fig. 4a, b), which demonstrates that the inoculation can be used regardless of the technological level of maize production system.

4.3. *Nitrogen fertilization*

Nitrogen fertilization is a key factor in the production of maize, but the production of N fertilizers is costly and it can harm the environment when applied in excess (Ahmed et al., 2017). In addition, the use efficiency of N fertilizers by plants is rarely higher than 50% relative to the amount applied to soils (Skowrońska and Filipek, 2014; Reetz, 2016; Sharma and Bali, 2018). Our results corroborate the study of Martins et al. (2018), who have stated that the inoculation of maize with Ab-V5 and Ab-V6 strains can be adopted aiming at the best use of N fertilizers. It also confirms that these strains are not incompatible with N fertilizers (Hungria, 2011; Hungria and Nogueira, 2019) as maize has responded positively to inoculation when grown under a wide range of N fertilizer rates (Fig. 4a,c). In agreement, Schmidt and Gaudin (2018) have also reported gains of maize grain yield after the inoculation with *Azospirillum* spp. independently on the N rate based on a global meta-analysis. This indicates that inoculation is a realistic option for sustainable intensification in maize cultivation, because it increases the use efficiency of N fertilizers. Despite it, further research is necessary to determine the threshold of N application reduction without negatively affecting yield. Such an evaluation could unveil the role of bacterial inoculation on maize efficiency for N use.

4.4. *Maize genotypes*

Brazil extends for 8,516,000 km^2 north-south orientated, presents diverse edaphoclimatic conditions, and dozens of cultivars have been grown in each of its several locations (Pereira Filho and Borghi, 2020). Therefore, it is of great importance that inoculants are efficient for a vast range of cultivars, which has been confirmed for this study (Fig. 5). It is also important to note that genetically modified cultivars of maize have responded to inoculation similarly to conventional cultivars (Fig. 5), and the lack of effect of maize transgeny has also been observed in studies with soybean co-inoculated with *A. brasilense* Ab-V5 and Ab-V6 (Barbosa et al., 2021). However, the magnitude of the beneficial effects on maize has varied according to the genetic modification event (Fig. 5), which may be related both to the genes and to the management related to the transgenic event.

Maize cultivars that hold the genetic modifications TC1507 x MON810 and MON89034 x TC1507 x NK603 have showed resistance to lepidopterans and herbicides (glufosinate and glufosinate+glyphosate, respectively), which may explain the greater response to inoculation than cultivars resistant to only lepidopterans (MON89034). There is better weed control when maize cultivars resistant to herbicides are grown, as there is the possibility of applying herbicide after plant emergence (Duke and Powles, 2009). This may be even more relevant in the case of glufosinate, as it presents a greater spectrum of control of weeds than glyphosate and a greater chance of keeping maize yield potential (Takano and Dayan, 2020). Maize cultivars with the genetic modification events TC1507 x MON810 and MON89034 x TC1507 x NK603 have also showed greater response to inoculation than cultivars with the TC1507 event (resistance to lepidopterans and glufosinate herbicide) (Fig. 5). The TC1507 genetic modification event is associated with the introduction of only one gene (cry1Fa2) related to resistance of lepidopterans attack, and the extensive use of maize cultivars holding this event has favored the spread of resistant insects in Brazil (Santos-Amaya et al., 2016; Eghrari et al., 2019) and other countries (Huang et al., 2014; Gutierrez-Moreno et al., 2020). On the other hand, cultivars holding the genetic events TC1507 x MON810 (genes cry1Fa2 and cry1Ab) and MON89034 x TC1507 x NK603 (genes cry1Fa2, cry1A.105, cp4epsps (aroA:CP4) and cry2Ab2) have more genes introduced for acquiring resistance to lepidopterans, which probably gives greater resistance to attack of insects.

4.5. *Edaphoclimatic factors*

The bacteria inoculation has provided significant increases in the grain yield of maize for all evaluated soil attributes (organic matter, texture, tillage management), and climate conditions (Fig. 6). This result is highly relevant, considering that maize is cultivated in several regions of Brazil under expressive variability of soils types and climate conditions. Although there was no difference in the effect of inoculation as a function of soil texture, further studies are needed to expand the number of samples in sandy soils. This is because plants grown on sandy soils are highly susceptibility to drought, and inoculation could favor plant water absorption, considering the increase in root system (Fig. 2) that has also been verified in a meta-analysis study on soybean (Barbosa et al., 2021).

The efficiency of maize has varied between subtropical (+7%) and tropical (+2.6%) climates (Fig. 6). Studies on Ab-V5 and Ab-V6 strains have initially been selected under subtropical conditions in southern Brazil (Hungria et al., 2010), and may exist higher adaptation of these strains to such a climate. However, it opposes what has been observed in a meta-analysis study on the co-inoculation of soybeans with *Bradyrhizobium* spp. and the same Ab-V5 and Ab-V6 strains of *A. brasilense* (Barbosa et al., 2021). These variations between plant species may be due to the contrasting response to climate, soil and cultivation conditions that may vary between the regions of subtropical and tropical climates in Brazil. Therefore, more detailed studies of each assay may indicate whether any factor prevails in subtropical conditions.

5. Conclusion

The benefits of inoculating maize with *Azospirillum brasilense* Ab-V5 and Ab-V6 strains have been highlighted in this meta-analysis study based on 103 field trials conducted at 54 sites in Brazil. The inoculation has significantly stimulated root development, which is decisive to increase grain yields and concentrations of N in leaves and grains. Consequently, the benefits of inoculation have not been restricted to quantitative effects, as they have also increased the nutritional value of maize for human and livestock consumption. Although the inoculation has favored grain yields of maize under diverse agronomic and edaphoclimatic conditions, it has been identified factors that can be used to increase inoculation efficiency. As an example, seed inoculation has been more efficient than leaf spray, and solid inoculant (peat) has performed better rather than the liquid one. When used as an alternative method to seed inoculation, particularly in the case of seeds treated with pesticides, inoculation should be applied at the initial vegetative stages of maize development (V2-V3). Considering that bacteria inoculation provides positive economic and environmental impacts, the technology should be widespread in order to increase yields and nutritional standards of maize. Finally, this study has shown that a program aimed at strain selection adapted to each country can raise crop yields and set sustainable strategies in agriculture.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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