Experimental Agriculture (2024), vol. 60, e9, 1–11
doi:10.1017/S0014479723000285

CAMBRIDGE UNIVERSITY PRESS

RESEARCH ARTICLE

Does coinoculation with bradyrhizobia and cyanobacteria improve groundnut growth and yield?

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(Received 30 May 2023; revised 26 September 2023; accepted 22 December 2023)

Summary
Groundnut plants can obtain N from N_2 fixation via symbiosis with rhizobia, and inoculation with selected strains can improve grain yields. We report the results of four field experiments carried out under subtropical conditions to confirm whether microbial inoculants can improve groundnut performance through the effects of single inoculation with *Bradyrhizobium arachidis* (SEMIA6144), coinoculation with *Arthrospira platensis* (IPR7059) or *Synechocystis* sp. (IPR7061), or N fertilization with 100 kg ha^{-1} N on plant growth, nodulation, N accumulation in tissues, grain protein concentration (GPC), and grain yield. There were no effects of inoculation treatment or N fertilizer on shoot or root dry weight. In clayey soil, coinoculation with *B. arachidis* and cyanobacteria increased grain productivity by an average of 19% compared to that in the noninoculated control. In this clayey soil with a higher P content, regardless of whether coinoculated with *B. arachidis* or cyanobacteria or single inoculated, grain productivity was 16% greater on average than that resulting from N fertilizer addition. In conclusion, the success of rhizobial inoculation in groundnuts is dependent on the soil, probably due to P limitation and weather conditions.

Keywords: Arthrospira platensis; Bradyrhizobium arachidis; Synechocystis sp.

Introduction

Groundnut (*Arachis hypogaea* L.) is a leguminous crop that produces grains inside shells that develop underground. This crop is highly valued for its oil (40–50%) and protein (20–30%) contents and is used for human food, livestock feed, and other industrial uses, including biodiesel production (Taurian et al., 2013; Moda-Cirino et al., 2015; Asante et al., 2020). Groundnut is currently cultivated worldwide in tropical, subtropical, and warm temperate regions, with the top five producers in tons of grain with shells being mainland China (18,307,800), India (10,244,000), Nigeria (4 607 669), the USA (2 898 140), and Sudan (2 355 000) (FAO, 2021). In the 2022/2023 crop season, Brazil produced 892 200 tons of grain, with an average productivity of 4,041 kg ha^{-1} (CONAB, 2023).

Groundnut plants have a high demand for N, which may result from biological N_2 fixation (BNF) (Kermah et al., 2018; Peoples et al., 2021). This process contributes to economic gains and plays an important role in maintaining soil fertility and the sustainability of agricultural production systems. Under field conditions, symbioses are established both with indigenous and cointroduced rhizobia strains (Muñoz et al., 2011; Bouznif et al., 2019) and may result in abundant nodulation (Castro et al., 1999; Lanier et al., 2005; Torres-Júnior et al., 2014; Asante et al., 2020; Jovino et al., 2022).

Bradyrhizobium spp. have been recognized as the most representative symbiotic rhizobial species for groundnuts (Bouznif et al., 2019); however, variable results have been reported. In the USA, inoculation was successful at increasing yields in only 7 out of 20 experiments in which groundnuts had not been previously grown (Lanier et al., 2005). In Argentina, inoculation of selected strains did not improve yields in comparison to those in noninoculated fields, and BNF by indigenous rhizobia allowed maximal yields to be reached (Bogino et al., 2006). For these examples and others elsewhere, the variable responses of the inoculation of groundnut with *Bradyrhizobium* could be attributed to the variable environmental conditions in which the crop was grown (Castro et al., 1999; Lanier et al., 2005; Bogino et al., 2006; Torres-Júnior et al., 2014; Asante et al., 2020; Jovino et al., 2022).

Moreover, eubacteria of the phylum Cyanobacteria have called the attention of producers seeking sustainable development (Sutherland et al., 2021; Taira et al., 2021) because they can be applied both for wastewater bioremediation (Araujo et al., 2021; Melo et al., 2022) and as agricultural inoculants, biofertilizers (Gavilanes et al., 2020; Horácio et al., 2020; Supraja et al., 2020), or foliar fertilizers (Amatussi et al., 2023). Cyanobacteria were the first organisms to evolve photosynthesis, and some species, such as *Anabaena* sp. and *Nostoc* sp., fix N_2. Additionally, they have been used as coinoculants with *Azospirillum brasilense* in maize (Gavilanes et al., 2020) and with a mix of *Rhizobium tropici* and *R. freirei* plus *A. brasilense* in common bean (Horácio et al., 2020). However, other cyanobacteria do not fix N but may contribute to crop growth via similar mechanisms to those of the most commonly known plant growth-promoting bacteria (Singh et al., 2017; Gavilanes et al., 2021).

In our study, two species of cyanobacteria were chosen based on previous work by our group that used groundnut in a field experiment (Andrade et al., 2014). The first, *Arthrospira* (*Spirulina*) *platensis*, is a filamentous cyanobacterium that grows under photoautotrophic conditions and does not fix N. When inoculated in *Phaseolus aureus* or *P. mungo* seeds (Bhowmik et al., 2010) or applied as a biofertilizer in *Begonia semperflorens* (Jowkar et al., 2017), rice (Dineshkumar et al., 2018), tomato (Supraja et al., 2020), or groundnut (Sivalingam, 2020), it increases seed germination and plant growth. The second species, *Synechocystis* sp., is also a unicellular nonnitrogen-fixing cyanobacterium that primarily inhabits water environments and soil and has been characterized by elevated production of phytohormones when inoculated in the rhizosphere of wheat (Khurshid et al., 2017).

To confirm whether microbial inoculants may improve groundnut performance, growth, and grain yield due to their beneficial effects, we carried out field experiments to compare N fertilizer with single inoculation and with double inoculation of *B. arachidis* with cyanobacteria.

Materials and Methods

Microbial strains

The *B. arachidis* strain (SEMIA6144, =SM400, =USDA3187, =MAR11), which is used in commercial inoculants in Brazil, was provided by the Microbiological Resource Center, Porto Alegre, Brazil (FEPAGRO). The cyanobacteria were obtained from the Microalgae IPR Collection of the Instituto de Desenvolvimento Rural do Paraná - IAPAR-EMATER (IDR-Paraná). *Arthrospira* (*Spirulina*) *platensis* (IPR7059) was kindly provided by Dr. Iracema de Oliveira Moraes (Fundação Andre Tosello, Brazil), and *Synechocystis* sp. (IPR7061) was isolated from freshwater in Paraná state, Brazil (Andrade et al., 2014).

Experimental site description and design

Field experiments were performed during the summer season of 2011–2012 at experimental stations in the IDR-Paraná under four different edaphic–climate conditions (Supplementary

Table 1. Soil physicochemical properties and most probable number (MPN) groundnut-nodulating of rhizobia (cells g of dry soil^{-1}) of soils and locations of the experimental fields. Soil sampling is done before sowing at top layer of 0 to 0.10 m. Values are on an oven-dried soil mass basis

Soil/climate features	Ponta Grossa	Paranavaí	Umuarama	Londrina
Sand (g kg^{-1})	160	880	850	230
Silt (g kg^{-1})	160	20	20	190
Clay (g kg^{-1})	680	100	130	580
pH ($CaCl_2$)	5.10	4.6	4.70	5.60
P (mg dm^{-3})	11.20	5.6	20.40	22.20
C (g dm^{-3})	25.78	5.37	9.11	16.51
N (g dm^{-3})	2.22	0.46	0.79	1.42
Al (cmol$_c$dm^{-3})	0.00	0.15	0,13	0.00
H + Al (cmol$_c$dm^{-3})	6.68	3.68	3.97	3.97
Ca (cmol$_c$dm^{-3})	4.52	1.77	1.67	4.80
Mg (cmol$_c$dm^{-3})	2.96	0.65	0.82	3.00
K (cmol$_c$dm^{-3})	0.12	0.10	0,12	0.50
S (cmol$_c$dm^{-3})	7.60	1.76	2.61	8.30
T (cmol$_c$dm^{-3})	14.28	5.43	6.58	12.27
V (%)	53.22	32.22	39.66	67.64
Al (%)	0.00	7.89	4.74	0.00
MPN Cells x10^3	1.12	9.17	3.57	7.23
Latitude	S 25º 05' 58''	23º04' S	S 23º 38' 51.8''	23° 18' 37'' S
Longitude	W 50º 09' 30''	52º 27' 56'' W	W 52º 42' 33.9''	51º 0.9' 46'' W
Elevation (m)	880	446	543	582
Köppen climate classification	*Cfb*	*Cfa*	*Cfa*	*Cfa*

Material Figure S1 available online at https://doi.org/10.1017/S0014479723000285 and Table 1) using two sandy soils (Paranavaí and Umuarama), a sandy-clayey–loamy soil (Ponta Grossa), and a clayey soil (Londrina), which are classified as Paleudult (udic Ultisol), Rhodic Haploperox (perudic Oxisol), and Rhodudult (udic Ultisol), respectively (Soil Survey Staff, 2014). The rainfall and temperature during the crop growing season at each experimental site are presented in Supplementary Material Figure S2a–d available online at https://doi.org/10.1017/S0014479723000285.

Soil chemical analyses were performed according to Pavan et al. (1992), and the soil total N content was calculated (Andrade et al., 2015) by using Equation 1:

$$N = C(1.724/20) \tag{1}$$

where N is the soil's total nitrogen and C is the soil's total carbon.

The most probable number (MPN) counts of fresh soil samples were performed using groundnut surface disinfected seeds pregerminated before planting in sterilized Leonard jars containing sterilized sand and vermiculite (3:1) and nutrient solution without N.

Before groundnut sowing, the soil was fertilized with 300 kg ha^{-1} 0–20–20 fertilizer, which was applied to the sowing furrows. The N fertilizer treatment involved 100 kg ha^{-1} N (urea), which was split into 20 kg N ha^{-1} applied in the furrow at sowing and 80 kg N ha^{-1} top-dressed on the soil surface at 25–30 d after emergence.

The treatments used were as follows: (i) control (without inoculation and N); (ii) N fertilizer with 100 kg ha^{-1} N; (iii) *B. arachidis* (peat); (iv) *B. arachidis* (fine shale); (v) *A. platensis* (fine shale); (vi) coinoculation with *B. arachidis* plus *A. platensis* (fine shale); (vii) *Synechocystis* sp. (fine shale); and (viii) coinoculation with *B. arachidis* plus *Synechocystis* sp. (fine shale). Inoculation of *B. arachidis* (SEMIA6144, =SM400, =USDA3187, =MAR11) was achieved by adding inoculant either to peat or fine shale (plus 1% (w/v) of carboxymethylcellulose and polyvinylpyrrolidone) as a solid carrier at a concentration of 10^9 viable cells g^{-1}. For inoculation, the seeds were moistened with sterile water supplemented with 10% sugar, after which the inoculant was applied to 50 kg of seeds.

A. platensis (IPR7059) and *Synechocystis* sp. (IPR7061) were grown in BG11 culture medium (Rippka et al., 1979) for 30 d using a 12-h light phase (temperature of 28.0 ± 2.0 °C) and a 12-h dark phase (22.0 ± 2.0 °C). Inoculation of the seeds with their respective treatments was performed using a suspension of cells of each of the cyanobacteria with approximately 10^6 cells mL^{-1} at a rate of 12 mL kg^{-1} of seeds. Sowing was performed manually in furrows made by disking, using an average of 60 kg seeds ha^{-1}. For weed control, trifluralin was applied one week before sowing according to the crop recommendations. The experiments were arranged in randomized complete blocks with five replications. Each plot had an area of 4.0 m × 6.0 m (24 m^2) and consisted of eight furrows with 6 m in length spaced 0.5 m apart.

Plant samplings

At late flowering (R2 stage), 8–12 plants from each plot were uprooted in the second and seventh rows to assess nodulation (nodule number and dry matter weight), shoot dry matter, and shoot N concentration. Nodulation was evaluated according to procedures described by Cardoso et al. (2009). Nodules and shoot dry weight were measured after drying at 60 °C for 72 h. For grain yield, the plants were harvested from the four central lines (8 m^2), and the results are expressed in kg ha^{-1} with moisture corrected to 8%.

The shoot N concentration and grain N concentration were analyzed by the Kjeldahl digestion method (Bremner and Keeney, 1966), and the shoot N uptake was calculated from the N concentration according to Horácio et al. (2020). Grain N accumulated (kg ha^{-1}) was calculated using grain N concentration multiplied by grain yield from each plot, and the crude protein concentration was calculated by multiplying the N content (%) by a factor of 6.25 (Jones, 1931). The shelling percentage (%) was calculated from the seed weight per plant relative to the pod weight multiplied by 100.

Statistical analysis

The data were analyzed for a normal distribution and homogeneity of variance and log-transformed when necessary. The data were analyzed by one-way analysis of variance according to the procedures outlined by Snedecor and Cochran (1980), and the means were compared by the Scott–Knott test ($p = 0.05$) using the statistical package SISVAR (Ferreira, 2011).

Results

Data from the soil chemical and rhizobia MPN analyses of the experimental areas are shown in Table 1. Tables 2–5 present data from the four experiments regarding plant development (dry weight of roots and shoots), shoot N concentration (SNC), nodulation (number and weight of nodules), shells and shelling, and N and grain protein concentration (%) of groundnut.

Discussion

There were no effects of inoculation treatment or N fertilizer on nodule weight or number in relation to the control, except in the sandy soils (Paranavaí and Umuarama) (Tables 2–5), indicating that naturalized bradyrhizobia was highly competitive in inducing nodule formation in groundnut.

Our nodulation data are in line with previous results obtained elsewhere in Brazil (Santos et al., 2017) and in Argentina (Castro et al., 1999; Bogino et al., 2006), the USA (Lanier et al., 2005), and Ghana (Asante et al., 2020). Most likely due to the promiscuous nature of groundnut plants and the high competitiveness of native rhizobial strains (Bouznif et al., 2019), only a few very effective strains stand out for their BNF rates and ability to promote plant growth (Bogino et al., 2006;

https://doi.org/10.1017/S0014479723000285 Published online by Cambridge University Press

Table 2. Roots dry weight (RDW), shoot dry weight (SDW), shoot N concentration (SNC) (g kg^{-1}), N uptake (mg N plant^{-1}), nodulation (nodule number and dry weight per plant), shell, shelling, grain N concentration (GNC) (g kg^{-1}), grain N accumulated (GNA) (kg ha^{-1}) and grain protein concentration (GPC) (%) of groundnut (cv. IAC Tatu-ST) inoculated with *Bradyrhizobium* sp. (SEMIA 6144), *Arthrospira platensis* (IPR7059) and *Synechocystis* sp. (IPR7061). Sandy–clayey–Loamy soil (Ponta Grossa, PR). Values are means of five repetitions

	RDW	SDW	SNC	N uptake	Nodule	Nodule	Shell	Shelling	GNC	GNA	GPC
Treatments	g pl^{-1}	g pl^{-1}	g kg^{-1}	mg pl^{-1}	Nº pl^{-1}	mg pl^{-1}	kg ha^{-1}	%	g kg^{-1}	kg ha^{-1}	%
Control	7.93	30.41	20.08†	604‡	7.9	34.0	1392	56	44.25	55	25.3
†N-fertilizer	6.66	29.66	18.64‡	557‡	10.4	25.3	1534	56	40.46	59	25.0
‡6144	9.44	30.08	20.65†	629‡	17.7	74.4	1560	57	40.92	60	25.6
§6144	7.79	33.38	16.48‡	547‡	18.0	57.6	1465	54	40.49	63	25.3
¶7059	6.33	32.44	18.53‡	598‡	11.7	43.9	1348	55	40.06	61	27.7
§7059 + 6144	8.88	37.13	19.20‡	726†	17.3	55.2	1262	57	42.36	49	26.4
¶7061	8.46	32.93	19.13‡	632‡	19.0	56.9	1323	56	42.22	54	27.1
§7061 + 6144	8.01	35.59	21.94†	764†	11.7	47.5	1460	59	43.36	52	26.5
F value	1.57ns	0.81ns	2.62*	2.69*	0.71ns	1.23ns	0.74ns	0.76ns	0.65ns	0.88ns	0.65ns
CV (%)	23.57	20.28	11.57	20.82	35.35	17.91	18.35	7.60	10.16	20.07	10.20

ns = not significant ($p > 0.05$); *Significant at ($p < 0.05$). Means followed by the same letter, within each column, are not different by Scott-Knott test, $p = 0.05$. †Urea (100 kg N ha^{-1}); ‡Peat or §Fine shale is used as carrier of microbial inoculants. ¶Cell suspension.

Table 3. Root dry weight (RDW), shoot dry weight (SDW), shoot N concentration (SNC) (g kg^{-1}), N uptake (mg N plant^{-1}), nodulation (nodule number and dry weight per plant), shell, shelling, grain N concentration (GNC) (g kg^{-1}), grain N accumulated (GNA) (kg ha^{-1}), and grain protein concentration (GPC) (%) of groundnut (cv. IAC Tatu-ST) inoculated with *Bradyrhizobium* sp. (SEMIA6144), *Arthrospira platensis* (IPR7059) and *Synechocystis* sp. (IPR7061). Sandy soil (Paranavaí, PR). Values are means of five repetitions

	RDW	SDW	SNC	N uptake	Nodule	Nodule	Shell	Shelling	GNC	GNA	GPC
Treatments	g pl^{-1}	g pl^{-1}	g kg^{-1}	mg pl^{-1}	Nº pl^{-1}	mg pl^{-1}	kg ha^{-1}	(%)	g kg^{-1}	kg ha^{-1}	%
Control	2.64†	26.22‡	28.17‡	678‡	179.6	96.13†	801	60	51.90	96†	33.1
†N-fertilizer	2.02†	24.95‡	32.60†	827†	145.2	65.30‡	851	60	54.63	98†	33.8
‡6144	2.67†	25.53‡	31.09†	812†	123.9	91.35†	1015	60	52.45	98†	32.8
§6144	2.13†	23.17‡	28.26‡	658‡	148.0	80.76†	999	63	52.91	100†	34.1
¶7059	1.41†	22.47‡	28.66‡	644‡	113.1	65.38‡	978	65	54.15	101†	32.4
§7059 + 6144	2.21†	31.37†	27.71‡	890†	105.2	69.15‡	820	63	53.12	83‡	33.0
¶7061	2.32†	25.37‡	32.26†	812†	149.0	86.63†	915	63	52.74	103†	32.8
§7061 + 6144	2.34†	21.93‡	34.31†	753†	100.4	57.33‡	963	60	52.53	106†	33.2
F value	2.77*	2.91*	2.37*	2.24*	1.43ns	3.14*	1.48ns	0.44ns	0.60ns	2.39*	0.59ns
CV (%)	24.28	15.56	12.60	17.83	18.08	23.29	16.81	8.36	4.96	11.84	4.97

ns = not significant ($p > 0.05$); *Significant at ($p < 0.05$). Means followed by the same letter, within each column, are not different by Scott-Knott test, $p = 0.05$. †Urea (100 kg N ha^{-1}); ‡Peat or §Fine shale is used as carrier of microbial inoculants. ¶Cell suspension.

Torres-Júnior et al., 2014; Jovino et al., 2022). The literature has noted that groundnut is a very promiscuous microsymbiont, as this crop establishes symbioses with both native and introduced rhizobia at the same rates (Torres-Júnior et al., 2014; Li et al., 2015; Muñoz et al., 2015; Bouznif et al., 2019).

Despite initial evidence of plant growth stimulation in other crops, in our study, there was a greater (but not significant) grain yield in the sandy soil (Paranavaí) due to coinoculation with *Synechocystis* sp. (IPR7061) and only significant effects on nodule weight in sandy soil (Umuarama) due to coinoculation of the *B. arachidis* strain (SEMIA6144) with *A. platensis* (IPR7059). In rhizobial inoculation, the stimulus from the cyanobacteria *A. platensis* (IPR7059) or *Synechocystis* sp. (IPR7061) or any other plant growth-promoting microorganism may not be the factor limiting the plant growth and grain yield of groundnut.

https://doi.org/10.1017/S0014479723000285 Published online by Cambridge University Press

Table 4. Roots dry weight (RDW), shoot dry weight (SDW), shoot N concentration (SNC) (g kg^{-1}), N uptake (mg N plant^{-1}), nodulation (nodule number and dry weight per plant), shell, shelling, grain N concentration (GNC) (g kg^{-1}), grain N accumulated (GNA) (kg ha^{-1}), and grain protein concentration (GPC) (%) of groundnut (cv. IAC Tatu-ST) inoculated with *Bradyrhizobium* sp. (SEMIA6144), *Arthrospira platensis* (IPR7059) and *Synechocystis* sp. (IPR7061). Sandy soil (Umuarama PR). Values are means of five repetitions

	RDW	SDW	SNC	N uptake	Nodule	Nodule	Shell	Shelling	GNC	GNA	GPC
Treatments	g pl^{-1}	g pl^{-1}	g kg^{-1}	mg pl^{-1}	Nº pl^{-1}	mg pl^{-1}	kg ha^{-1}	%	g kg^{-1}	kg N ha^{-1}	%
Control	2.22	15.07	30.70	454	99.7	116.6§	2157	63	54.44	201	34.5
†N-fertilizer	2.11	16.11	29.22	471	110.9	122.0§	2124	65	54.88	217	34.8
‡6144	2.28	15.06	29.42	442	48.9	93.5§	2088	68	55.38	244	34.6
§6144	2.02	13.70	30.26	413	63.7	110.2§	2139	65	55.27	216	34.3
¶7059	2.24	17.03	29.30	513	97.9	124.3§	2201	63	55.72	201	34.0
§7059 + 6144	2.43	18.30	29.87	545	169.7	216.2†	2087	65	55.43	198	33.8
¶7061	2.32	18.18	31.61	566	97.2	161.7‡	2169	62	54.09	207	34.7
§7061 + 6144	2.28	15.27	32.80	499	86.2	146.4§	1996	64	55.58	198	34.6
F value	0.47ns	0.73ns	0.44ns	0.70ns	1.94ns	5.68**	0.15ns	0.94ns	0.48ns	1.46ns	0.49ns
CV (%)	18.38	26.49	13.97	28.75	29.39	26.51	20.32	7.31	3.39	13.79	3.40

ns = not significant ($p > 0.05$); *Significant at ($p < 0.05$), **Significant at ($p < 0.01$). Means followed by the same letter, within each column, are not different by Scott-Knott test, $p = 0.05$. †Urea (100 kg N ha^{-1}); ‡Peat or §Fine shale is used as carrier of microbial inoculants. ¶Cell suspension.

Table 5. Roots dry mass (RDW), shoot dry mass (SDW), shoot N concentration (SNC) (g kg^{-1}), N uptake (mg N plant^{-1}), nodulation (nodule number and dry weight per plant), shell, shelling, grain N concentration (GNC) (g kg^{-1}), grain N accumulated (GNA) (kg ha^{-1}), and grain protein concentration (GPC) (%) of groundnut (cv. IAC Tatu-ST) inoculated with *Bradyrhizobium* sp. (SEMIA6144), *Arthrospira platensis* (IPR7059) and *Synechocystis* sp. (IPR7061). Clayey soil (Londrina, PR). Values are means of five repetitions

	RDW	SDW	SNC	N uptake	Nodule	Nodule	Shell	Shelling	GNC	GNA	GPC
Treatments	g pl^{-1}	g pl^{-1}	g kg^{-1}	mg pl^{-1}	Nº pl^{-1}	mg pl^{-1}	kg ha^{-1}	%	g kg^{-1}	kg ha^{-1}	%
Control	5.69	16.68	35.31	582	52.6	39.32	1798	64	49.49	151‡	30.9
†N-mineral	4.27	15.98	32.61	522	32.4	31.42	1879	64	48.00	158‡	30.0
‡6144	5.13	16.45	32.80	537	37.0	43.85	1932	67	49.29	197†	30.8
§6144	4.25	13.85	36.61	508	42.4	41.72	1823	68	49.52	190†	31.0
¶7059	5.68	16.42	34.02	555	36.6	43.38	1852	68	47.88	185†	29.9
§7059 + 6144	4.37	14.49	34.80	505	41.3	42.09	1848	67	49.86	189†	31.2
¶7061	5.82	15.14	34.95	532	49.6	51.92	1864	68	48.84	190†	30.5
§7061 + 6144	4.92	15.27	34.81	537	36.9	45.40	1701	67	50.17	176†	31.4
F value	1.22ns	0.16ns	0.30ns	0.15ns	0.98ns	1.38ns	0.14ns	0.44ns	0.83ns	2.89*	0.83ns
CV (%)	27.08	22.42	15.74	26.54	38.02	25.92	21.68	8.36	4.16	12.21	4.16

ns = not significant ($p > 0.05$); *Significant at ($p < 0.05$). Means followed by the same letter, within each column, are not different by Scott-Knott test, $p = 0.05$. †Urea (100 kg N ha^{-1}); ‡Peat or §Fine shale is used as carrier of microbial inoculants. ¶Cell suspension.

In the sandy-clayey–loamy soil (Ponta Grossa), there was a response to single inoculation with *B. arachidis* (SEMIA6144), in which the yield increased by 14%–24%, and to N fertilizer (urea), with a 20% increase, showing that yield was limited by rhizobial inoculation or N fertilizer (Figure 1a). In the clayey soil (Londrina), single inoculation with *B. arachidis* (SEMIA6144) or *Synechocystis* sp. (IPR7061) or coinoculation with *B. arachidis* and *A. platensis* (IPR7059) increased the amount of N accumulated in the grains, which was greater than that in the noninoculated and N-fertilized soils (Table 5), indicating that BNF is essential for grain yield in these soils (Figure 1d).

Coinoculation of rhizobia with other microorganisms has often been suggested as an additive strategy for improving rhizobial symbiosis and BNF in grain legumes due to complementary mechanisms of plant growth promotion (Ahemad and Kibret, 2014; Gavilanes et al., 2020). There have been several successful examples of coinoculation of groundnut with *Bradyrhizobium* and

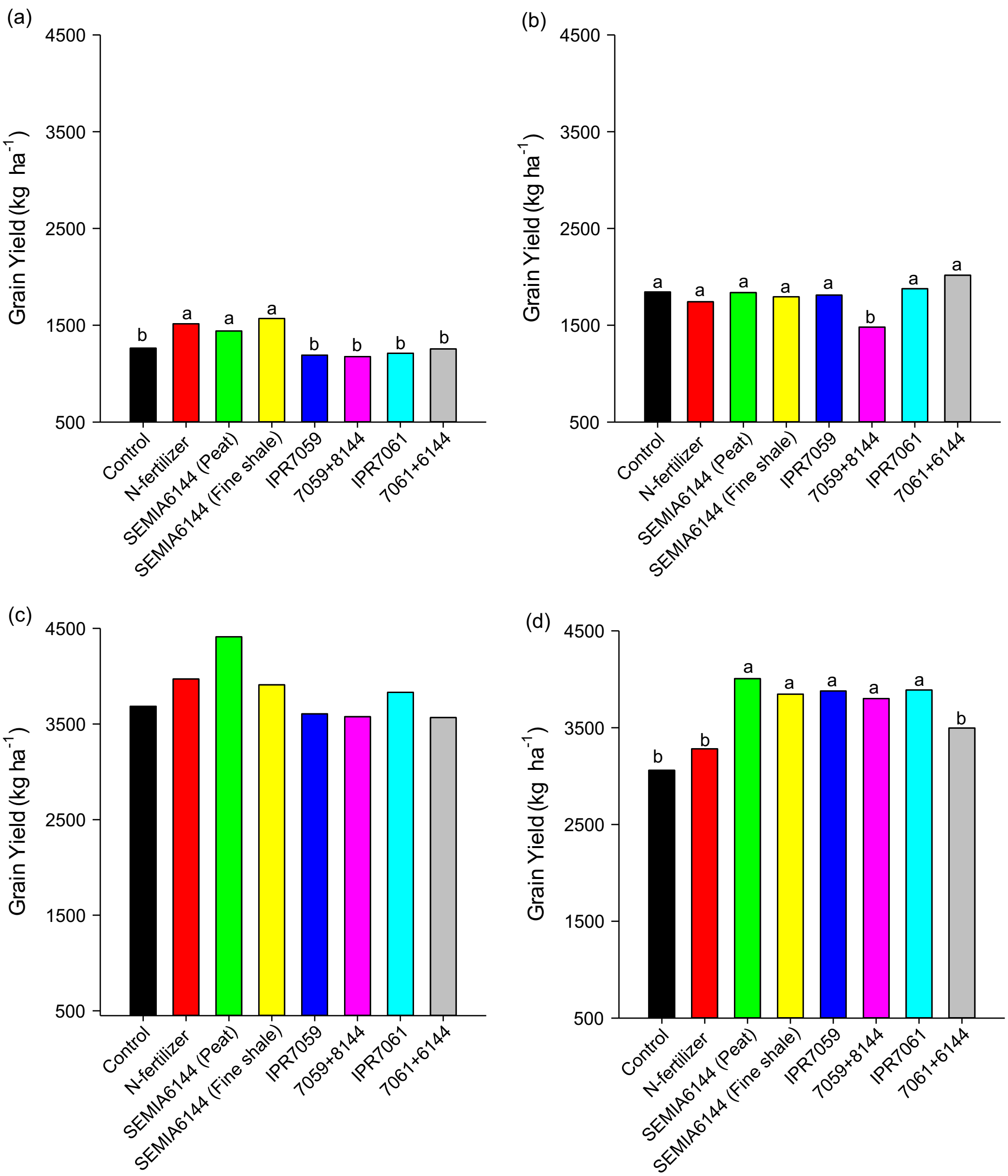

Figure 1. Grain yield (kg ha^{-1}) of groundnut (cv. IAC Tatu-ST) seeds inoculated with *Bradyrhizobium* sp. (SEMIA6144), *Arthrospira platensis* (IPR7059) and *Synechocystis* sp. (IPR7061). Cropping seasons 2011/2012. (a) Sandy-clayey–loamy soil (Ponta Grossa), (b) sandy soil (Paranavaí), (c) sandy soil (Umuarama), and (d) clayey soil (Londrina). The bars are the means of five repetitions, and the same letter indicates that the data were not significantly different according to the Scott–Knott test ($p = 0.05$). The N fertilizer (100 kg N ha^{-1}) was urea.

other collaborative microorganisms, e.g., *Azospirillum brasilense* (Gericó et al., 2020), *Bacillus* spp. (Figueredo et al., 2014; Preyanga et al., 2021; Kaschuk et al., 2022), *Serratia marcescens*, and *Trichoderma harzianum* (Badawi et al., 2011). The benefits of coinoculation may be direct on nodule formation or indirect; for example, a meta-analysis revealed that coinoculation of bradyrhizobia and *Bacillus* spp. significantly increases the sizes of roots and shoots of groundnut (Kaschuk et al., 2022).

https://doi.org/10.1017/S0014479723000285 Published online by Cambridge University Press

Among grain legumes, groundnut, and common bean are the crops with the highest gap in BNF to meet their N demands (Palmero et al., 2022), whereas crops such as soybean promptly respond to inoculation with elite strains and obtain sufficient N_2 fixation to satisfy their N demands (Kaschuk et al., 2016).

Studies on the introduction of efficient *Bradyrhizobium* strains have shown that it is a limiting factor for nodule efficiency and plant growth in groundnut. Inoculation with *B. arachidis* (SEMIA6144) resulted a greater grain yield compared to the control, similar to the effects of both N fertilizer (urea) and selected efficient *Rhizobium* strains isolated from Brazilian soils, for instance, the 23M isolate (Santos et al., 2017) and the *Bradyrhizobium* sp. strain ESA123 (Jovino et al., 2022).

In our study, the cyanobacteria IPR7059 and IPR7061 were chosen to be coinoculated with *B. arachidis* (SEMIA6144) because these genera are related to plant growth in other crops (Bhowmik et al., 2010; Khurshid et al., 2017; Sivalingam, 2020; Supraja et al., 2020). According to Liebig's law of the minimum, crop yield is limited by the lack of one or more factors that are present in lesser quantities. Usually, N is one of the most limiting factors for grain legume crops. However, under the conditions of our experiments, nodulation and BNF were not the limiting factors for groundnut yield. Moreover, interestingly, indicators of plant growth (root and shoot dry weights, shoot N concentration (SNC), shells, and shelling) hardly changed because of the inoculation treatments, but their averages varied among the different locations. In fact, studies have indicated that the performance of rhizobial symbioses in groundnut is influenced by cultivar, rhizobial strain, soil physical and chemical attributes, land use history, and weather conditions (e.g., Lanier et al. 2005, Bogino et al. 2006, Santos et al. 2008, Torres-Junior et al. 2014, Santos et al. 2017, Asante et al. 2020).

Considering the different locations (soil and climate), the average yield of groundnut was much lower in the sandy-clayey–loamy soil (Ponta Grossa) (1852 kg ha^{-1}) and in the sandy soil (Paranavaí) (1328 kg ha^{-1}) (Figure 1a and b) and were comparable to the values in lesser productive areas in the world, which includes the national average of Brazil (FAO, 2021). However, the grain yield in the other sandy soil (Umuarama) was 3819 kg ha^{-1}, and that in the clayey soil (Londrina) was 3657 kg ha^{-1} (Figure 1c and d); these values are comparable to the average yields of the most productive countries, for example, Argentina, with 3455 kg ha^{-1} in 2019 (FAO, 2021), and Brazil (Moda-Cirino et al., 2015; CONAB, 2023).

The lower grain yields in Paranavaí and Ponta Grossa than in Londrina and Umuarama may be due to the lower amount of rainfall during the stages in which the plants needed water for development (Supplementary Material Figure S2a–d available online at https://doi.org/10.1017/S0014479723000285). Additionally, when analyzing the soil chemical characteristics of the field experiments, it was found that the two locations with higher grain yields (Umuarama and Londrina) had greater soil phosphorus (P) availability than did Paranavaí and Ponta Grossa (Table 1). In these two field experiments, lower soil P content should be highlighted as a possible cause of lower groundnut yields since it is an important factor for symbiosis. In our experiments, these findings provide evidence that soil P availability may be limiting groundnut.

Overall, the naturalized rhizobial population capable of nodulating groundnut, which ranged from 1.12 to 9.8×10^3 per g of soil, did not appear to influence the response to inoculation with bradyrhizobia.

In Londrina, where the soil P supply was greater (Table 1), the inoculation of *B. arachidis* (SEMIA6144) increased yields in comparison to those in the noninoculated and N-fertilized treatments (Figure 3d). This result suggested that P limitation is a possible explanation for the slight effects of rhizobial inoculation, although the experiment was not designed to test this hypothesis. Indeed, in Ghana, the influence of P fertilization on groundnut yields was demonstrated by Asante et al. (2020) using the USDA3456 and BR3267 strains and by Yaro et al. (2021) using the BR3267 strain.

Hence, we conclude that coinoculation of *B. arachidis* with the cyanobacteria *A. platensis* (IPR7059) or *Synechocystis* sp. (IPR7061) overcame the limitations for increasing nodulation, BNF, and grain yield in groundnut in only one out of four field experiments, where the weather and soil P content conditions were better for the crop. The success of groundnut inoculation depends on the interaction of soil factors (e.g., P) and weather conditions. Subsequent studies of rhizobial inoculation should include P fertilization and consortia with microorganisms that increase the availability of P (Taurian et al., 2013). In our study, the lack of response of groundnut to N fertilization in increasing grain yield should be highlighted, which indicates the importance of inoculation with selected strains of *Bradyrhizobium*, as this crop has also been included in rotation with sugarcane to improve soil fertility and is also used in the food industry.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0014479723000285

Availability of data and materials. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgements. The authors are grateful to Maria Aparecida de Matos, Paulo S. Aguilar, Alisson W. Sanzovo, Aretusa D. R. Mendes, and Cezar F. Araujo Junior. M.H. and D.S. Andrade (315060/2020-4) acknowledge the research fellowships received from CNPq.

Authors' contributions. All the authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by DSA and GML. The first draft of the manuscript was written by DSA, GK, and MH. All the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Financial support. This study was partially supported by Petrobras SIX, São Mateus do Sul, PR – Agricultural Shale Project at Instituto Agronômico do Paraná (IAPAR) and by the CNPq (National Council for Scientific and Technological Development, Brazil), Project INCT-CNPq (MPCPAgro 465133/2014-2) and Fundação Araucária (SETI-043/2019).

Competing interests. 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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Cite this article: Andrade DS, Lovato GM, Kaschuk G, and Hungria M. Does coinoculation with bradyrhizobia and cyanobacteria improve groundnut growth and yield?. *Experimental Agriculture*. https://doi.org/10.1017/S0014479723000285