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Native *Bradyrhizobium* Enhancing the Growth of Two Peruvian Commercial Varieties of Cowpea

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ABSTRACT

Background: *Bradyrhizobium* establishes an effective symbiosis with cowpea. This study evaluated the inoculation of seven native strains in two commercial varieties, demonstrating their potential to enhance symbiotic nitrogen fixation (SNF) and contribute to the agronomic utilization of cowpea in degraded soils of the Peruvian arid coast.

Methods: We implemented a completely randomized design with ten treatments: seven inoculated with selected *Bradyrhizobium* strains-four *B. yuanmingense* (8.1.1, 8.2, 19.3, 20.1), two *Bradyrhizobium* sp. (1.1, 12.3) and one *B. diversitatis* 25.1 and three controls-a consortium of effective strains (*B. yuanmingense* Rc-458-01 and Rc-455-02), a fertilized treatment (40:60:30; N: P: K) and an uninoculated, unfertilized absolute control. Reactivated and purified strains were used to prepare inocula. Two cowpea varieties (Vaina verde INIA-432 and Vaina verde CAR-9) were sown in non-sterile soil and inoculated with standardized cell suspensions. We performed two evaluations: At 45 days post-inoculation, we assessed symbiotic effectiveness through plant height, shoot and root dry weight, nodule dry weight, number of nodules, shoot nitrogen content and nitrogen accumulation; at maturity, we evaluated yield and grain quality, including grain nitrogen, phosphorus and potassium content, number of pods per plant, 100-seed weight, grains per pod and grain yield per plant.

Result: Inoculation with native *B. yuanmingense* strains 19.3 and 20.1, as well as *Bradyrhizobium* sp. 12.3, significantly increased symbiotic and grain-related parameters in both cowpea varieties.

Key words: *Bradyrhizobium yuanmingense*, Cowpea variety, Effectiveness, Grain weight per plant.

INTRODUCTION

The use of inoculants to improve grain legume yield and quality is a key strategy for sustainable agriculture, reducing reliance on synthetic nitrogen fertilizers (Rani *et al.,* 2019). This practice Supports Development Goals (SDGs) 2, 13 and 15 by enhancing food security, climate resilience and biodiversity (Stagnari *et al.,* 2017). Although nitrogen is essential for plant growth, excessive use of synthetic fertilizers negatively impacts ecosystems and human health (Ahmed *et al.,* 2017). Furthermore, recent geopolitical disruptions have affected global fertilizer availability (Hailes *et al.,* 2023). These issues underscore the importance of Biological Nitrogen Fixation through legume-rhizobia symbiosis as a sustainable alternative.

Cowpea, a grain legume of African origin, plays a vital economic and environmental role due to its resilience to drought, heat and poor soils (Narayana and Angamuthu, 2021; Pavithra *et al.,* 2025), making it suitable for arid, climate-affected regions like coastal Peru (Murga-Orrillo *et al.,* 2024). In 2023, global production reached 9.78×10^6 tons, with Peru contributing 3.21×10^4 tons (FAO, 2025). Although cowpea is a promiscuous legume, forming symbioses with diverse rhizobia, studies show specificity at species, strain and varietal levels. Effective symbionts include *Bradyrhizobium yuanmingense*, *B. liaoningense* (Valdez *et al.,* 2016), *B. pachyrhizi* (Leite *et al.,* 2018), *B. uaiense* (Cabral-Michel *et al.,* 2020), *B. amazonense* (Moreira *et al.,* 2024), among others.

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Bradyrhizobium-based inoculants significantly enhance symbiotic efficiency and agronomic performance in cowpea under controlled conditions (Valdez *et al.,* 2016; Araújo *et al.,* 2017). Inoculation effectiveness depends on chemical (pH, salinity, nutrient availability), physical (soil texture, compaction) and biological factors, including native rhizobia presence, inoculant strain efficacy and cowpea genotype. Cowpea varieties vary in their Biological Nitrogen Fixation (BNF) capacity, influenced by environmental factors such as phosphorus and water availability (Jemo *et al.,* 2017). Genetic regulation of BNF involves dominant-recessive epistatic genes (Seido *et al.,* 2019). Mohammed *et al.* (2020) reported BNF efficiencies ranging from 27% to 97% among Ghanaian cultivars.

In Peru, rhizobial inoculation research in cowpea is limited. Valdez *et al.* (2016) identified effective *Bradyrhizobium* strains from floodplain soils in Ucayali-two *B. yuanmingense* and two *B. liaoningense*-that increased grain yield by 29.5% compared to the control and by 6.3% over nitrogen fertilization (60 kg N ha^{-1}). However, no rhizobial strains are currently recommended for cowpea in Peru's arid coastal zones. Evaluating Biological Nitrogen Fixation (BNF) potential in commercial varieties across agroecological zones is crucial, accounting for genotype-rhizobium-environment interactions (Seido *et al.,* 2019). We hypothesize that strains adapted to arid coastal conditions will exhibit superior symbiotic performance.

This study presents the first evaluation of the symbiotic and agronomic potential of seven native strains from three *Bradyrhizobium* species, effective in symbiosis with cowpea and isolated from the arid coastal zone of Peru. The objectives were: (1) to assess the symbiotic effectiveness of seven selected *Bradyrhizobium* strains under non-sterile soil conditions; and (2) to evaluate the influence of inoculating these strains on the agronomic performance of two commercial cowpea varieties under the same soil conditions.

MATERIALS AND METHODS

Origin of rhizobia strains and cowpea varieties

The *Bradyrhizobium* strains were previously isolated from the arid northern and central coast of Peru and selected them based on their symbiotic effectiveness, osmotolerance and salt tolerance by Valdez-Nuñez *et al.* (2025). The strains are currently preserved in the Collection of Beneficial Microorganisms for Agriculture and Livestock at Universidad Nacional de Barranca (ColMiBAP-UNAB). The strains were reactivated from cryovials stored at -80°C and cultured them on YEM agar at 28°C for 7 days. We evaluated two cowpea varieties: Vaina Verde INIA-432, a commercially demanded cultivar provided by the National Institute of Agrarian Innovation (INIA, 2013) and Vaina Verde CAR-9, an experimental line donated by Granos y Legumbres-Peru.

Experimental design under controlled conditions

The study, conducted from January to May 2024, evaluated the symbiotic efficiency of seven *Bradyrhizobium* strains using a completely randomized 10 $\times$ 2 factorial design. Twenty treatments with eight replicates assessed nitrogen fixation in two cowpea varieties at two growth stages, 45 days after sowing and the remaining at grain maturity.

The soil used in this study was collected from the Buena Vista experimental field at the Universidad Nacional de Barranca (UNAB), Peru (10°452′47.5″S; 77°442′21″W; 64 m.a.s.l.). The soil, with a history of sugarcane (*Saccharum officinarum*) cultivation and no prior rhizobia inoculation. Soil was taken from the top 20 cm, air-dried, ground, sieved (4 mm mesh) and placed (4 kg) into 5 kg-capacity pots. An aliquot was analyzed at the Soil Laboratory of Universidad Nacional Agraria La Molina for texture and chemical composition, including pH, EC (1:1), $CaCO_3$, OM, N, P, K, CEC, exchangeable cations (Ca^{2+}, Mg^{2+}, K^{2+}, Na^{2+}) and micronutrients (Cu^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, B^{+}) (Supplementary Table 1). All treatments received uniform phosphorus and potassium fertilization (60:30 kg ha^{-1}) using 1250 mg triple superphosphate and 150 mg potassium chloride per pot.

Quantification of rhizobial numbers in the experimental soil

The native rhizobial population capable of nodulating cowpea was quantified using the Most Probable Number (MPN) technique (Somasegaran and Hoben, 2012). Eight decimal dilutions (10^{-1} to 10^{-8}) were prepared from 10 g of soil in 9 mL of 10 mM $MgCl_2$, homogenized with a vortex mixer (Scientific Industries, USA). Surface-disinfected seeds of cowpea variety Vaina Verde INIA-432 were germinated following Valdez *et al.* (2016) and sown individually in growth pouches containing nitrogen-free Jensen's solution. Each pouch received a specific soil dilution, with four replicates per dilution. Pouches were incubated at 25±

Supplementary Table 1: Physical and chemical characterization of the soil from the Buena vista experimental campus, District and Province of Barranca, used in the pot experiment.

pH (1:1)	CIC	C.E. (1:1)	P	K	Cu	Mn	Fe	Zn	B	Total of cations and bases
			Available							
		dS/m	ppm							
7.41	4.16	0.66	13.3	89	3.5	11.87	27.17	2.25	1.34	4.16
$CaCO_3$	OM	Total N	Base	Ca^{+2}	Mg^{+2}	K^{+}	Na^{+}	Sand	Lime	Clay
		%	saturation	meq/100 g					g Kg^{-1}	
0.72	0.82	0.05	100	3.29	0.59	0.17	0.10	740	150	110

CEC: Cation exchange capacity; EC: Electrical conductivity; OM: Organic matter; Total N: Total nitrogen.

2°C with a 12/12 h photoperiod for 21 days. Nodulation was assessed by nodule presence and MPN values were estimated using probability tables Somasegaran and Hoben (2012).

Inoculum preparation and standardization

The strains were reactivated in 3 mL YEM broth tubes, incubated for 5 days at 150 rpm and 28±1°C on an orbital shaker (MRC, Israel). Subsequently, each strain was transferred to 50 mL flasks containing 27 mL of YEM broth and incubated them under the same conditions for 7 days. Each culture was standardized an OD_{600} ~ 1.0 using 10 mM $MgCl_2$. For the consortium treatment, four standardized inocula were mixed in equal ratios (1:1:1:1). Cell concentrations were quantified via the drop plate method (Ferreira *et al.,* 2024).

Experimental setup

Seeds of cowpea varieties Vaina Verde INIA-432 and CAR-9 were selected and surface-disinfecting them with 70% ethanol (1 min), 2.5% sodium hypochlorite (5 min) and rinsing them eight times with sterile distilled water. Six seeds per pot were sowed and thinned to three plants five days post-emergence. Each seed received 1/ mL of standardized inoculum based on the treatment. To maintain uniform moisture, they calculated the water-holding capacity (WHC) and maintained it at 60% by monitoring pot weight. Irrigation used only distilled water. Control treatments (T9 and T10) received 1 mL of 10 mM $MgCl_2$. The experiment was conducted on metal tables in a greenhouse from Buena Vista experimental field at the Universidad Nacional de Barranca (UNAB), Peru (10°45′55.7″S; 77°44′27.6″W; 64/ m.a.s.l.), where temperatures ranged from 23°C to 30°C.

Experimental evaluation

At 45-50 days after sowing, when 50% of treatments reached flowering, were conducted the first evaluation. Four plants were harvested per treatment to assess plant height (H, cm), shoot dry weight (SDW, mg) and root dry weight (RDW, mg), with biomass dried at 70°C for 72 hours (Valdez *et al.,* 2016). Nodulation was assessed using a 0-4 scale based on nodule number, size and position and nodules on the root crown (NNC) were counted following Yates *et al.* (2016). Nodule dry weight (NDW, mg) was recorded and shoot nitrogen content (SNC, %) was determined by the Kjeldahl method (Rocha *et al.,* 2021). Shoot nitrogen accumulation (SNA, mg N plant^{-1}) was calculated as SDW $\times$ SNC% (Florentino *et al.,* 2010). Symbiotic efficiency (EFI) and effectiveness (EFV) were determined relative to controls (Valdez *et al.,* 2016) and categorized according to Purcino *et al.* (2000): Highly effective (EFV > 80%), effective (50-79%), slightly effective (35-49%), or ineffective (<34%). At maturity, remaining plants were harvested to assess agronomic traits: number of pods per plant (NPP), number of grains per pod (NGP), 100-seed weight (W100), grain weight per plant (GWP) and grain nutrient content (N-G, P-G, K-G%).

Statistical analysis

The statistical analyses were conducted using InfoStat software (Di Rienzo *et al.,* 2020). The assumptions of

A: Uninoculated control without nitrogen fertilization, B: Control with nitrogen fertilization (40 kg N ha-1), C: Inoculating consortium, D: *B. yuanmingense* 20.1, E: *Bradyrhizobium sp.* 12.3, F: *B. yuanmingense* 19.3.

Fig 1: Cowpea plants of the variety Vaina verde INIA-432 at 45 days after sowing.

normality and homoscedasticity was verified for all variables. Nodulation data (PSN, NNC and nodulation score) were transformed using the formula $(x + 0.5)^{0.5}$. One-way ANOVA was applied at a significance level of $P<0.05$, followed by Scott-Knott or Kruskal-Wallis tests. Pearson's correlation coefficients were used to assess relationships between vegetative and grain yield parameters.

RESULTS AND DISCUSSION

Inoculation with *Bradyrhizobium* strains significantly enhanced cowpea varieties by increasing H, RDW, symbiotic efficiency (EFI, EFV), GWP and %P-G. Inoculated treatments consistently outperformed the uninoculated controls (Fig 1 and 2, Table 1 and Table 4). Native *Bradyrhizobium* strains effectively fix atmospheric nitrogen in symbiosis with cowpea (Kyei-Boahen *et al.,* 2017; Nguyen *et al.,* 2020). Understanding both the genetic affiliation of rhizobial strains and the genotype of the host cultivar is crucial for selecting rhizobia that optimize grain yield, fertilizer use efficiency and tolerance to edaphoclimatic stress (Mendoza-Suárez *et al.,* 2020).

The H did not show significant differences among inoculated treatments within each cowpea variety. However, *B. yuanmingense* strains 19.3 and 20.1 increased H by 32.1% in INIA-432 and 40.6% in CAR-9, compared to the uninoculated control. H showed a strong correlation with grain yield in INIA-432 (r = 0.72**). *Bradyrhizobium* sp. 12.3 achieved the highest increase in shoot dry weight (SDW), with a 151.1% rise in INIA-432, while *B. yuanmingense* 8.2 increased SDW by 97.8% in CAR-9. Similarly, *B. diversitatis* 25.1 increased root dry weight (RDW) by 127.1% in INIA-432 and *B. yuanmingense* 8.2 increased RDW by 79.3% in CAR-9 relative to the controls. SDW reflects rhizobial symbiotic effectiveness and depends on the nitrogen source, the cultivar and their interaction (Fageria *et al.,* 2014).

In our study, shoot nitrogen content (SNC) and shoot nitrogen accumulation (SNA) were consistently lower in CAR-9 than in INIA-432. Nitrogen-fertilized treatments showed higher SNC and SNA than most inoculated treatments in both varieties. However, in CAR-9, inoculation with *Bradyrhizobium* sp. 12.3, *B. yuanmingense* 8.1.1, 20.1, 19.3; *B. diversitatis* 25.1 and the consortium produced SNC

Table 1: Experimental details.

Nitrogen treatments	Cowpea varieties Vaina verde INIA-432 Vaina verde CAR-9
T1	*Bradyrhizobium* sp. 1.1 (UNAB000121)
T2	*B. yuanmingense* 8.1.1 (UNAB000145)
T3	*B. yuanmingense* 8.2 (UNAB000147)
T4	*Bradyrhizobium* sp. 12.3 (UNAB000158)
T5	*B. yuanmingense* 19.3 (UNAB000258)
T6	*B. yuanmingense* 20.1 (UNAB000258)
T7	*B. diversitatis* 25.1 (UNAB000265)
T8	Commercial inoculant consortium (*B. yuanmingense* Rc-458-01 and Rc-455-02)
T9	Nitrogen-fertilized control (210 mg urea pot^{-1}, equivalent to 40 kg N ha^{-1})
T10	Uninoculated and unfertilized control

A: Uninoculated control without nitrogen fertilization, B: Control with nitrogen fertilization (40 kg N ha^{-1}), C: Inoculating consortium, D: *B. yuanmingense* 20.1, E: *Bradyrhizobium* sp. 12.3, F: *B. yuanmingense* 19.3.

Fig 2: Cowpea plants of the variety Vaina verde CAR-9 at 45 days after sowing.

Table 2: Average values of plant height (H), shoot dry weight (SDW), root dry weight (RDW), shoot nitrogen content (SNC), shoot nitrogen accumulated (SNA), efficiency (EFI) and effectiveness (EFV) obtained from two cowpea varieties treated with different nitrogen sources under mesh house conditions.

Var	Nitrogen source	H	SDW	RDW	SNC	SNA	EFI	EFV
		cm	mg plant $^{-1}$		%	mg N plant $^{-1}$	%	
Vaina verde INIA-432	1.1	21.38 (±0.84) Ba	5248.55 (±186.68) Aa	1911.93 (±46.34) Bb	2.07 (±0.01) Cd	108.64 (±0.64) Cd	234.61 (±4.10) Bb	67.34 (±0.92) Cb
	8.1.1	23.55 (±0.96) Ab	5036.75 (±392.62) Aa	1932.33 (±118.96) Bb	2.19 (±0.11) Cc	110.43 (±2.22) Cc	238.41 (±5.30) Bb	68.43 (±1.53) Cd
	8.2	23.46 (±0.81) Ab	5259.69 (±94.16) Aa	2133.00 (±53.13) Bb	2.13 (±0.01) Cc	111.90 (±0.99) Cc	241.54 (±1.84) Bb	69.36 (±1.31) Cd
	12.3	21.24 (±0.87) Ba	5521.90 (±140.11) Aa	2181.97 (±71.89) Bb	2.19 (±0.01) Cc	121.07 (±1.28) Bb	261.43 (±5.54) Aa	75.00 (±0.25) Bd
	19.3	26.35 (±1.35) Aa	5109.58 (±170.72) Aa	2091.80 (±65.77) Bb	2.33 (±0.02) Bb	119.18 (±3.24) Bb	257.53 (±9.61) Aa	73.82 (±1.70) Bd
	20.1	24.81 (±0.93) Aa	5000.39 (±205.35) Aa	1998.60 (±31.59) Bb	2.30 (±0.08) Bb	115.14 (±2.92) Cc	248.50 (±6.05) Bb	71.32 (±1.50) Cd
	25.1	24.05 (±1.47) Ab	5263.14 (±264.95) Aa	2284.97 (±86.00) Aa	2.32 (±0.00) Bb	122.23 (±0.54) Bb	263.94 (±3.85) Aa	75.75 (±0.79) Bd
	CCI	25.53 (±1.43) Aa	5066.13 (±216.81) Aa	2074.60 (±145.03) Bb	2.27 (±0.03) Bb	115.00 (±4.49) Cc	248.31 (±10.23) Bb	71.19 (±2.08) Cd
	CFN	26.09 (±1.54) Aa	6068.87 (±377.11) Aa	2541.13 (±81.73) Aa	2.66 (±0.09) Aa	161.43 (±1.98) Aa	-	100.00 (±0.00) Aa
	CA	19.95 (±1.00) Bb	2198.80 (±106.67) Bb	1006.10 (±60.00) Cd	2.11 (±0.01) Cc	46.34 (±0.63) De	100.00 (±0.00) Ce	28.73 (±0.67) Df
	C.V (%)*	13.82	9.51	7.10	3.99	4.02	5.18	3.55
Vaina verde CAR-9	1.1	25.16 (±1.81) Aa	4954.57 (±210.04) Ba	1595.57 (±32.52) Ac	1.99 (±0.01) Bd	98.60 (±0.61) Cd	183.66 (±2.34) Bd	79.99 (±3.19) Bc
	8.1.1	27.04 (±1.10) Aa	5274.32 (±140.71) Aa	1651.53 (±46.87) Ac	2.18 (±0.04) Ac	115.11 (±5.72) Bc	214.55 (±11.73) Ac	93.49 (±6.65) Ab
	8.2	27.43 (±0.32) Aa	5365.21 (±87.68) Aa	1690.30 (±34.33) Ac	1.93 (±0.02) Bd	103.68 (±0.77) Cd	193.13 (±2.40) Bd	84.11 (±3.38) Bc
	12.3	28.00 (±1.81) Aa	5205.52 (±254.19) Aa	1652.47 (±75.37) Ac	2.21 (±0.02) Ac	115.04 (±0.64) Bc	214.30 (±2.60) Ac	93.27 (±3.24) Ab
	19.3	26.01 (±3.12) Aa	5145.49 (±224.33) Aa	1555.50 (±80.32) Ac	2.19 (±0.06) Ac	112.82 (±0.88) Bc	210.12 (±2.12) Ac	91.50 (±3.53) Ab
	20.1	28.65 (±3.34) Aa	4918.96 (±139.33) Ba	1572.63 (±60.48) Ac	2.28 (±0.06) Ab	112.03 (±3.94) Bc	208.65 (±7.40) Ac	91.18 (±6.31) Ab
	25.1	26.78 (±2.24) Aa	4713.88 (±131.48) Ba	1578.20 (±59.71) Ac	2.17 (±0.01) Ac	102.17 (±0.23) Cd	190.31 (±1.41) Bd	82.85 (±2.91) Bc
	CCI	22.01 (±1.32) Bb	4627.93 (±92.33) Ba	1499.90 (±64.42) Ac	2.15 (±0.09) Ac	99.62 (±1.39) Cd	185.59 (±3.76) Bd	80.75 (±2.87) Bc
	CFN	23.30 (±1.27) Bb	5332.26 (±246.70) Aa	1578.53 (±105.84) Ac	2.32 (±0.03) Ab	123.84 (±4.85) Ab	-	100.00 (±0.00) Aa
	CA	20.38 (±0.46) Bb	2712.00 (±80.04) Cb	942.53 (±56.01) Bd	1.98 (±0.03) Bd	53.70 (±0.38) De	100.00 (±0.00) Ce	43.56 (±1.67) Ce
	C.V (%) *	21.45	7.17	7.35	4.98	5.32	5.65	9.15
	C.V (%) **	18.33	8.46	7.26	4.48	4.66	5.28	7.42

Different capital letters indicate significant differences between treatments at the cowpea variety level and lowercase letters indicate significant differences between cowpea varieties. Analysis of variance was performed using the Scot-Knott test with a 95% confidence interval. Var: Variety; CCI: Inoculant Consortium Control Treatment; CFN: Control treatment fertilized with mineral nitrogen (40 kg N ha^{-1}); CA: Absolute control treatment without inoculum and without nitrogen fertilization. C.V (%) *: Coefficient of variation obtained for each variety; C.V (%) **: Coefficient of variation of the data obtained in both varieties.

values statistically comparable to the fertilized control. Genotype-dependent differences in symbiotic performance are highlighted (Jemo *et al.,* 2017), considering that the SNA serves as a robust indicator of symbiotic effectiveness, which depends on both biotic and abiotic factors, including strain-host compatibility (Florentino *et al.,* 2010).

B. diversitatis 25.1 increased SNA by 163.8% in INIA-432 and *B. yuanmingense* 8.1.1 increased it by 114.4% in CAR-9. The poor SNA in control treatments suggests low native rhizobial effectiveness, likely due to their low soil density (<10^2 cells g^{-1}). Low soil nitrogen (0.05%) did not hinder symbiosis, allowing inoculated strains to perform efficiently and contribute significantly to biological nitrogen fixation and nitrogen accumulation (Nguyen *et al.,* 2020).

We modified the formulas from Purcino *et al.* (2000) to calculate EFI and EFV, using SNA instead of SDW, as a more accurate measure of symbiotic performance. Future comparative studies should validate this approach. In our study, EFI values in INIA-432 were significantly higher than those in CAR-9, likely due to genotypic differences between varieties. Our native *Bradyrhizobium* strains doubled EFI in all INIA-432 treatments and in at least 50% of CAR-9 treatments, highlighting the impact of host genotype on symbiotic effectiveness (Mohammed *et al.,* 2020).

Similarly, EFV was higher in CAR-9, where all inoculated treatments-except *Bradyrhizobium* sp. 1.1-were highly effective in BNF (Table 2). Notably, *B. yuanmingense* strains 8.1.1, 19.3 and 20.1 performed well in both varieties. In addition, native rhizobia in control treatments formed few, poorly located nodules due to late, inefficient infections. Although, consortium inoculation reduced nodulation compared to single strains, *B. diversitatis* 25.1 enhanced nodule dry weight (NDW) in both varieties (Table 3). These differences may reflect limitations in the nitrogen-fixing capacity of the selected genotypes (Graham *et al.,* 2004).

At harvest, all agronomic yield parameters-except N-G% and K-G%-were higher in inoculated treatments than in the non-inoculated control without nitrogen fertilization. Inoculation with *Bradyrhizobium yuanmingense* 20.1 increased the NPP by 39.2% in CAR-9 and 64.03% in INIA-432. Likewise, *Bradyrhizobium* sp. 12.3 improved the NGP by 25.2% (CAR-9) and 86.3% (INIA-432). Strains 12.3, 19.3

Table 3: Average values of nodular dry weight (NDW), number of nodules in crown (NNC) and nodulation score obtained from two cowpea varieties treated with different nitrogen sources under net house conditions.

Var	Nitrogen source	NDW mg $plant^{-1}$	NNC Nodules $plant^{-1}$	Nodulation score
Vaina verde INIA-432	1.1	139.35 (±8.44) Cc	4.88 (±1.01) Cd	2.00 (±0.00) Bc
	8.1.1	191.49 (±6.70) Bb	7.88 (±1.51) Bc	2.25 (±0.16) Bc
	8.2	196.14 (±6.59) Bb	9.38 (±2.17) Bc	2.50 (±0.27) Bb
	12.3	160.50 (±8.60) Bc	11.00 (±1.21) Bc	2.63 (±0.18) Bb
	19.3	172.14 (±6.03) Bc	12.38 (±2.24) Bc	2.50 (±0.27) Bb
	20.1	172.10 (±6.84) Bc	20.50 (±1.43) Aa	3.50 (±0.19) Aa
	25.1	237.18 (±23.92) Aa	22.13 (±1.92) Aa	3.50 (±0,19) Aa
	CCI	206.53 (±9.82) Ab	17.50 (±2.45) Ab	3.25 (±0.16) Aa
	CFN	126.73 (±12.03) Cd	0.37 (±0.18) De	1.63 (±0.18) Cd
	CA	66.43 (±8.17) De	0.38 (±0.18) De	1.13 (±0.13) Cd
	C.V (%)	13.11	20.43	21.29
Vaina verde CAR-9	1.1	127.54 (±9.52) Cd	12.38 (±2.50) Ac	2.75 (±0.25) Aa
	8.1.1	151.64 (±11.74) Bc	16.25 (±3.60) Ab	3.00 (±0.27) Aa
	8.2	161.73 (±5.05) Bc	15.75 (±1.44) Ab	3.25 (±0.16) Aa
	12.3	149.37 (±8.17) Bc	16.00 (±3.27) Ab	3.00 (±0.27) Aa
	19.3	138.15 (±7.34) Bc	12.13 (±1.27) Ac	2.63 (±0.18) Ab
	20.1	148.43 (±4.04) Bc	21.00 (±2.29) Aa	3.25 (±0.16) Aa
	25.1	190.07 (±4.01) Ab	14.50 (±1.38) Ab	2.75 (±0.16) Aa
	CCI	179.51 (±11.50) Ab	16.25 (±1.68) Ab	3.13 (±0.13) Aa
	CFN	80.41 (±12.84) De	3.50 (±0.87) Bd	1.25 (±0.16) Bd
	CA	101.13 (±11.01) Dd	2.63 (±0.42) Bd	1.88 (±0.13) Bc
	C.V (%) *	12.72	20.96	20.44
	C.V (%) **	6.69	20.79	20.85

Different capital letters indicate significant differences between treatments at the cowpea variety level, and lowercase letters indicate significant differences between cowpea varieties. Analysis of variance was performed using the Scot-Knott test with a 95% confidence interval. Var: Variety; CCI: Inoculant Consortium Control Treatment; CFN: Control treatment fertilized with mineral nitrogen (40 kg N ha^{-1}); CA: Absolute control treatment without inoculum and without nitrogen fertilization. C.V (%) *: Coefficient of variation obtained for each variety; C.V (%) **: Coefficient of variation of the data obtained in both varieties.

Table 4: Average values of number of pods per plant (NPP), number of grain per pod (NGP), 100 seed weight (W100), grain weight per plant (GWP), mineral contents in grain (N, P and K) obtained from two cowpea varieties treated with different nitrogen sources under mesh house conditions.

VAR	Fuente de nitrógeno	NPP	NGP	W100	GWP	N-G	P-G	K-G
		Number plant^{-1}			g plant^{-1}		%	
Vaina verde	1.1	3.56 (±0.08) Cc	7.08 (±0.47) Ab	30.44 (±0.32) Aa	12.26 (±0.24) Ac	3.40 (±0.01) Ab	0.39 (±0.01) Aa	1.06 (±0.03) Aa
INIA-432	8.1.1	4.22 (±0.08) Bb	7.17 (±0.59) Ab	28.92 (±0.63) Bb	12.81 (±0.13) Ac	3.38 (±0.06) Ab	0.41 (±0.01) Aa	1.09 (±0.00) Aa
	8.2	3.44 (±0.21) Cc	7.83 (±0.39) Ab	29.85 (±0.58) Ab	11.71 (±0.26) Ac	3.39 (±0.07) Ab	0.40 (±0.01) Aa	1.09 (±0.02) Aa
	12.3	4.33 (±0.27) Bb	9.00 (±0.99) Aa	29.07 (±0.31) Bb	14.73 (±1.86) Ac	3.39 (±0.02) Ab	0.39 (±0.01) Aa	1.08 (±0.01) Aa
	19.3	4.11 (±0.08) Bb	7.92 (±0.43) Ab	30.83 (±0.51) Aa	14.39 (±0.40) Ac	3.46 (±0.02) Aa	0.37 (±0.01) Aa	1.07 (±0.02) Aa
	20.1	4.56 (±0.21) Ab	7.75 (±0.52) Ab	29.43 (±0.51) Ab	15.35 (±2.01) Ab	3.51 (±0.01) Aa	0.40 (±0.01) Aa	1.06 (±0.03) Aa
	25.1	4.00 (±0.00) Bc	7.42 (±0.36) Ab	28.51 (±0.64) Bc	13.31 (±0.86) Ac	3.46 (±0.04) Aa	0.38 (±0.01) Aa	1.08 (±0.01) Aa
	CCI	4.56 (±0.08) Ab	7.42 (±0.31) Ab	29.69 (±0.38) Ab	13.91 (±0.28) Ac	3.47 (±0.04) Aa	0.37 (±0.03) Aa	1.05 (±0.02) Aa
	CFN	4.67 (±0.27) Ab	7.67 (±0.28) Ab	27.76 (±0.31) Bc	17.22 (±0.85) Aa	3.45 (±0.01) Aa	0.39 (±0.02) Aa	1.09 (±0.02) Aa
	CA	2.78 (±0.08) Dd	4.83 (±0.32) Bc	24.61 (±0.37) Cd	7.02 (±0.65) Bd	3.45 (±0.03) Aa	0.33 (±0.02) Ab	1.06 (±0.01) Aa
	C.V (%)	8.09	23.86	3.27	12.76	1.88	6.16	3.01
Vaina verde	1.1	5.11 (±0.34) Aa	8.00 (±0.17) Bb	29.56 (±0.30) Ab	18.13 (±0.52) Aa	3.26 (±0.01) Ac	0.32 (±0.00) Ab	1.05 (±0.03) Aa
CAR-9	8.1.1	4.78 (±0.16) Aa	8.17 (±0.39) Aa	27.68 (±0.33) Bc	17.86 (±0.24) Aa	3.28 (±0.07) Ac	0.33 (±0.01) Ab	1.06 (±0.01) Aa
	8.2	4.44 (±0.08) Ab	8.58 (±0.31) Aa	28.17 (±0.44) Bc	16.40 (±0.36) Bb	3.38 (±0.03) Ab	0.33 (±0.00) Ab	1.08 (±0.01) Aa
	12.3	4.56 (±0.08) Ab	9.08 (±0.48) Aa	27.33 (±0.55) Bc	18.41 (±0.58) Aa	3.30 (±0.05) Ac	0.34 (±0.01) Ab	1.10 (±0.01) Aa
	19.3	4.67 (±0.24) Ab	7.58 (±0.23) Bb	28.49 (±0.07) Bc	18.64 (±0.60) Aa	3.43 (±0.02) Aa	0.34 (±0.01) Ab	1.06 (±0.01) Aa
	20.1	5.11 (±0.21) Aa	8.50 (±0.45) Aa	28.19 (±0.12) Bc	18.34 (±0.25) Aa	3.36 (±0.03) Ab	0.32 (±0.00) Ab	1.07 (±0.01) Aa
	25.1	4.89 (±0.16) Aa	7.50 (±0.23) Bb	27.63 (±0.11) Bc	17.92 (±0.78) Aa	3.29 (±0.01) Ac	0.31 (±0.00) Ab	1.05 (±0.01) Aa
	CCI	4.78 (±0.21) Aa	8.42 (±0.29) Aa	27.77 (±0.52) Bc	18.56 (±0.55) Aa	3.37 (±0.04) Ab	0.34 (±0.01) Ab	1.08 (±0.02) Aa
	CFN	4.56 (±0.16) Ab	8.50 (±0.31) Aa	28.18 (±0.24) Bc	17.74 (±0.14) Aa	3.36 (±0.03) Ab	0.34 (±0.01) Ab	1.08 (±0.00) Aa
	CA	3.67 (±0.14) Bc	7.25 (±0.25) Bb	26.97 (±0.14) Bc	15.31 (±0.08) Bb	3.32 (±0.01) Ac	0.26 (±0.01) Bc	1.02 (±0.01) Aa
	C.V (%) *	8.21	13.86	2.34	4.53	1.89	3.95	2.25
	C.V (%) **	8.18	19.06	2.86	8.58	1.89	5.37	2.66

Different capital letters indicate significant differences between treatments at the cowpea variety level, and lowercase letters indicate significant differences between cowpea varieties. Analysis of variance was performed using the Scot-Knott test with a 95% confidence interval. Var: Variety; CCI: Inoculant Consortium Control Treatment; CFN: Control treatment fertilized with mineral nitrogen (40 kg N ha^{-1}); CA: Absolute control treatment without inoculum and without nitrogen fertilization. C.V (%) *: Coefficient of variation obtained for each variety; C.V (%). **: Coefficient of variation of the data obtained in both varieties.

Table 5: Correlation coefficients between height (H), Shoot dry weight (SDW), Root dry weight (RDW), Shoot nitrogen content (SNC), Shoot nitrogen accumulated (SNA), Effectiveness (EFV), Nodule dry weight (NDW), Number of nodules in crown (NNC), Number of pods per plant (NPP), Number of grains per plant (NGP), Grain weight per plant (GWP), P content in grain (P) in two varieties of cowpea (Vaina verde INIA 432 / Vaina verde CAR 9).

	H	SDW	RDW	SNC	SNA	EFV	PSN	NNC	NPP	NGP	GWP	P
H	1.00											
SDW	0.58* / 0.69**	1.00										
RDW	0.67** / 0.76**/	0.97*** 0.98***	1.00									
SNC	0.74** / 0.24^{ns}	0.48^{ns} / 0.39^{ns}	0.63** / 0.32^{ns}	1.00								
SNA	0.70** / 0.63** /	0.95*** 0.95***	0.97*** / 0.90***	0.73** / 0.67**	1.00							
EFV	0.70 **/ 0.64**	0.95*** / 0.95***	0.97*** / 0.90***	0.73** / 0.67**	1.00*** / 1.00***	1.00						
PSN	0.50^{ns} / 0.45^{ns}	0.59* / 0.25^{ns}	0.61** / 0.42^{ns}	0.06^{ns} /- 0.08^{ns}	0.46^{ns} / 0.15^{ns}	0.46^{ns} / 0.16^{ns}	1.00					
NNC	0.39^{ns} / 0.75**	0.28^{ns} / 0.51^{ns}	0.33^{ns} / 0.63**	0.01^{ns} / 0.14^{ns}	0.19^{ns} / 0.44^{ns}	0.19^{ns} /0.45^{ns}	0.80** / 0.79**	1.00				
NPP	0.72** / 0.58*	0.74** / 0.68**	0.76** / 0.75**	0.67** / 0.39^{ns}	0.81** / 0.67**	0.80** / 0.69**	0.47^{ns} / 0.44^{ns}	0.43^{ns} / 0.68**	1.00			
NGP	0.43^{ns} / 0.43^{ns}	0.87*** / 0.62**	0.83*** / 0.62**	0.28^{ns} / 0.31^{ns}	0.77** / 0.61**	0.77** / 0.62**	0.57* / 0.09^{ns}	0.42^{ns} / 0.45^{ns}	0.70** /0.30^{ns}	1.00		
GWP	0.72** / 0.43^{ns}	0.88*** / 0.66**	0.89*** / 0.67**	0.73** / 0.64**	0.94*** / 0.75**	0.94*** / 0.75**	0.41^{ns} / 0.36^{ns}	0.32^{ns} / 0.55^{ns}	0.93*** / 0.82***	0.80** / 0.36^{ns}	1.00	
P	0.30^{ns} / 0.46^{ns}	0.78** / 0.91***	0.67** / 0.86***	0.13^{ns} / 0.48^{ns}	0.65** / 0.91***	0.65** / 0.90***	0.54^{ns} / 0.25^{ns}	0.25^{ns} / 0.46^{ns}	0.54^{ns} / 0.60*	0.69** / 0.67**	0.61** / 0.77**	1.00

and 20.1 showed consistent effects in increasing the GWP across both cowpea varieties. In INIA-432, strain 20.1 outperformed both the inoculant consortium and the uninoculated control, increasing GWP by 98.2% (CAR-9) and 118.7% (INIA-432). These results highlight the superior symbiotic efficiency of strain 20.1 under nitrogen-deficient conditions (Table 4).

Pearson correlation coefficients (Table 5) revealed significant positive relationships between vegetative and yield parameters in both cowpea varieties. Strong correlations were observed between SDW and RDW (0.98***), as well as between SNA and EFV (0.95***). SNA also showed a highly significant correlation with GWP in INIA-432 (0.94***) and CAR-9 (0.75***), confirming its relevance as an indicator of symbiotic efficiency (Araújo *et al.,* 2017; Kyei-Boahen *et al.,* 2017).

In contrast, the correlation between SDW and NDW was weak in INIA-432 and non-significant in CAR-9, likely due to soil limitations such as texture and low phosphorus availability (Rocha *et al.,* 2021). The P-G% was strongly correlated with vegetative traits-SDW, RDW, SNA, EFV- and moderately correlated with yield components-NGP and GWP. Phosphorus is essential for photosynthesis, ATP synthesis and nitrogen fixation, emphasizing its critical role in promoting effective legume-rhizobia symbiosis and cowpea productivity (Araújo *et al.,* 2017; Mobeena *et al.,* 2025).

B. yuanmingense is a widespread symbiont of cultivated cowpea across Asia, Africa and South America (Valdez *et al.,* 2016; Leite *et al.,* 2018; Ndungu *et al.,* 2018; Girija *et al.,* 2020). Its high nitrogen-fixing efficiency, as demonstrated in strains like BR3267 (Leite *et al.,* 2018). In our study, *B. yuanmingense* strains 19.3 and 20.1, along with the undefined species *Bradyrhizobium* sp. 12.3, showed strong symbiotic performance in both cowpea varieties. These strains, isolated from arid Peruvian regions, possess key plant growth-promoting traits such as auxin and siderophore production, phosphate solubilization and stress tolerance (Valdez-Nuñez *et al.,* 2025). They represent promising inoculant candidates for arid agroecosystems, but require field validation across diverse environments and farmer-oriented implementation strategies.

CONCLUSION

The strains studied enhanced the agronomic performance of both commercial cowpea varieties. However, the Vaina Verde CAR-9 variety demonstrated greater symbiotic potential for BNF, particularly in terms of EFV and GWP. *Bradyrhizobium yuanmingense* strains 19.3 and 20.1, along with *Bradyrhizobium* sp. 12.3, were selected for both cowpea varieties due to their ability to promote SNA and GWP. These strains outperformed currently recommended inoculants, making them promising candidates for the formulation and production of strain-specific inoculants adapted to the arid coast of Peru.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

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Conflict of interest

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