FREE LIVING NITROGEN FIXING PLANT GROWTH PROMOTING *RHIZOBACTERIA*: ECOLOGICAL ROLES AND APPLICATIONS

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Abstract

Plant roots are inhabited by a large diversity of microbes, some of which are beneficial for the growth of plants and known as plant growth promoting rhizobacteria (PGPR). Free-living and associative nitrogen-fixing plant growth-promoting rhizobacteria (PGPR) represent a promising sustainable alternative to chemical fertilizers, by supplying biologically fixed nitrogen (N_2) and multiple plant growth-enhancing activities. Members of *Azospirillum*, *Azotobacter*, *Herbaspirillum seropedicae*, *Burkholderia*, *Pseudomonas*, and *Bacillus* have been demonstrated to fix nitrogen under non-symbiotic or weakly symbiotic associations with non-leguminous crops, produce phytohormones, solubilize phosphorus, suppress pathogens, and improve stress tolerance. Through these multifaceted interactions, PGPR not only improve nutrient uptake but also bolster plant defenses, contributing to healthier and more resilient crops. Despite many promising findings, challenges remain in accurately determining the efficiency of nitrogen fixation, owing to the immense complexity of soil biome interactions and the diverse mechanisms through which bacteria influence plant physiology. In this article, we aim to provide a practical comparison to help farmers understand how they can effectively reduce chemical fertilizer usage by applying PGPR inoculants.

Keywords: *Rhizobacteria***,** nitrogen fixing.

JEL: Q19.

Introduction

1. Free-Living Nitrogen-Fixing PGPR

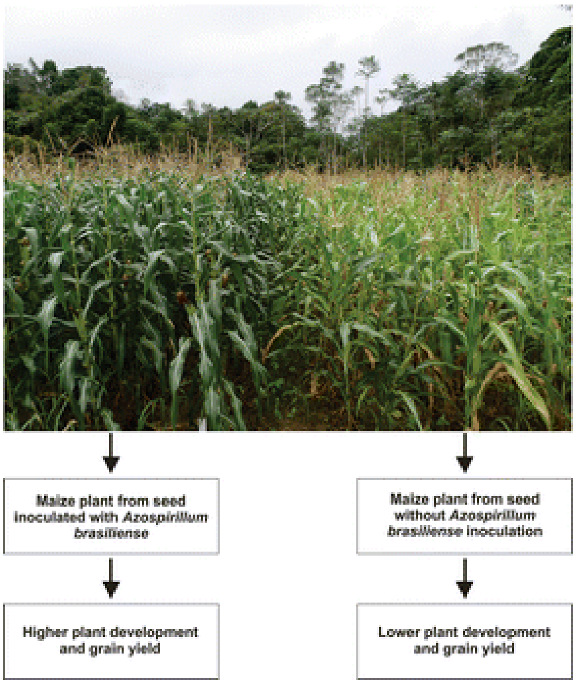
Free-living and associative nitrogen-fixing plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative to chemical fertilizers by supplying biologically fixed nitrogen and enhancing plant growth through various mechanisms. Understanding the characteristics and applications of these bacteria is essential for farmers seeking to optimize crop yields while reducing chemical inputs.

Free-living nitrogen fixation (FLNF) in the rhizosphere, or N fixation by heterotrophic bacteria living on/near root surfaces, is ubiquitous and a significant source of N in some terrestrial systems. FLNF is also of interest in crop production as an alternative to chemical fertilizer, potentially reducing production costs and ameliorating negative environmental impacts of fertilizer N additions [18].

1.1. Azospirillum sp.

Azospirillum species are well-documented for their associative nitrogen fixation with cereals such as maize, wheat, and rice. They produce phytohormones like auxins and cytokinins, which promote root development and enhance nutrient uptake. Inoculation with *Azospirillum* has been shown to increase crop yields by up to 30% under field conditions, particularly in nitrogen-deficient soils [5] (Fig. 1).

Multispecies inocula combining nitrogen fixers and other PGPR (e.g., *Azospirillum*, *Bacillus*) can further boost plant growth and N_2 fixation, surpassing effects of single strains [4] (Fig. 2).



Source: [8].

Fig. 1. Maize inoculated with *Azospirillum* left and control right

1.2. Azotobacter sp.

Azotobacter species are obligate aerobes capable of free-living nitrogen fixation. They produce exopolysaccharides, vitamins, and growth-promoting substances that improve soil structure and stimulate plant growth. Field trials have

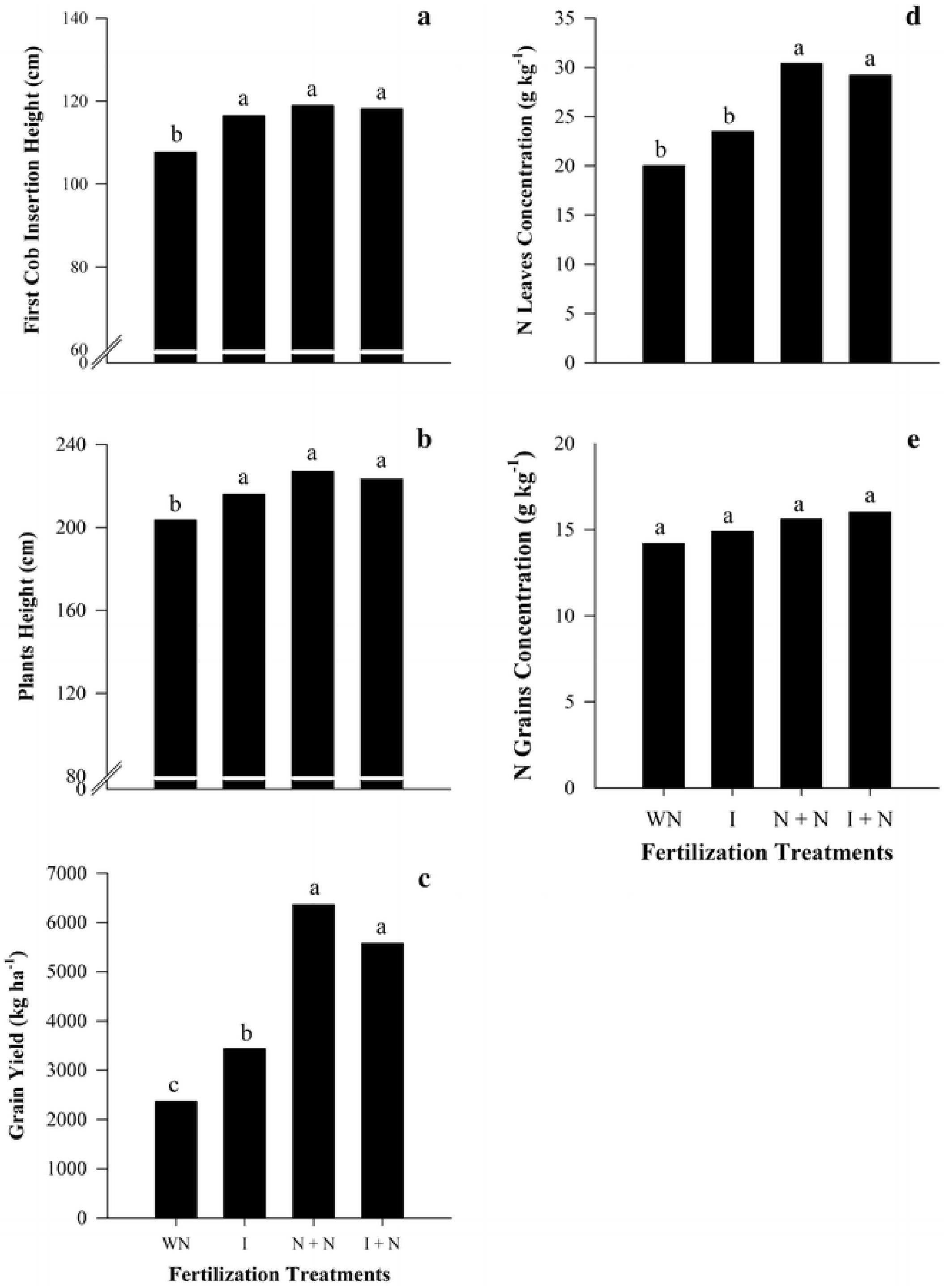


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Fig. 2. Effects of N fertilization treatments (N), with and without inoculation with *Azospirillum brasiliense* (I)

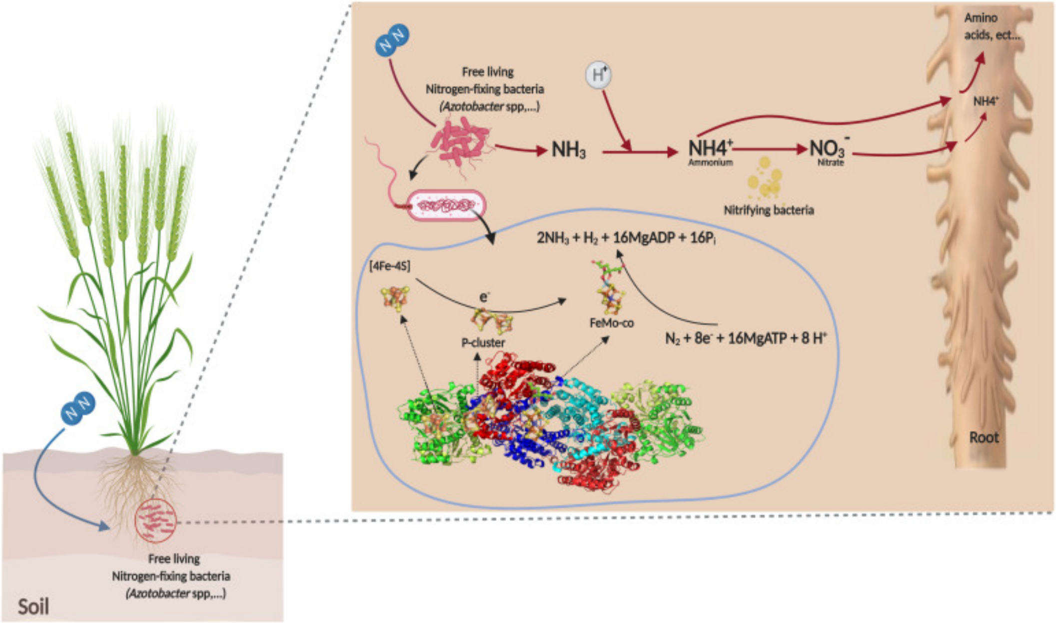

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Fig. 3. Mechanism of non-symbiotic fixation of atmospheric nitrogen by *Azotobacter* sp.

demonstrated that *Azotobacter* inoculation can increase crop yields by 15–20%, making it a viable option for reducing synthetic fertilizer use [2].

Two-weeks old primed (*Azotobacter vinelandii*) and un-primed plants were exposed to 0, 50, 150, and 250 mM NaCl for 15 days. *Azotobacter* primed maize plants (40% and 80%) expressed substantial improvements in plant growth and photosynthetic efficiency due to sustainable nitrogen assimilation under saline soil. Osmotic adjustments under salt stress were enhanced in 40% primed plants than un-primed stress plants. Microbial primed plants expressed lesser hydrogen per oxide (H_2O_2) and *Malondialdehyde* (MDA) production, higher Performance index (PI), and improved effective quantum yield of PSII photochemistry (ΦPSII) under saline soil [20] (Fig. 4).

1.3. Burkholderia sp.

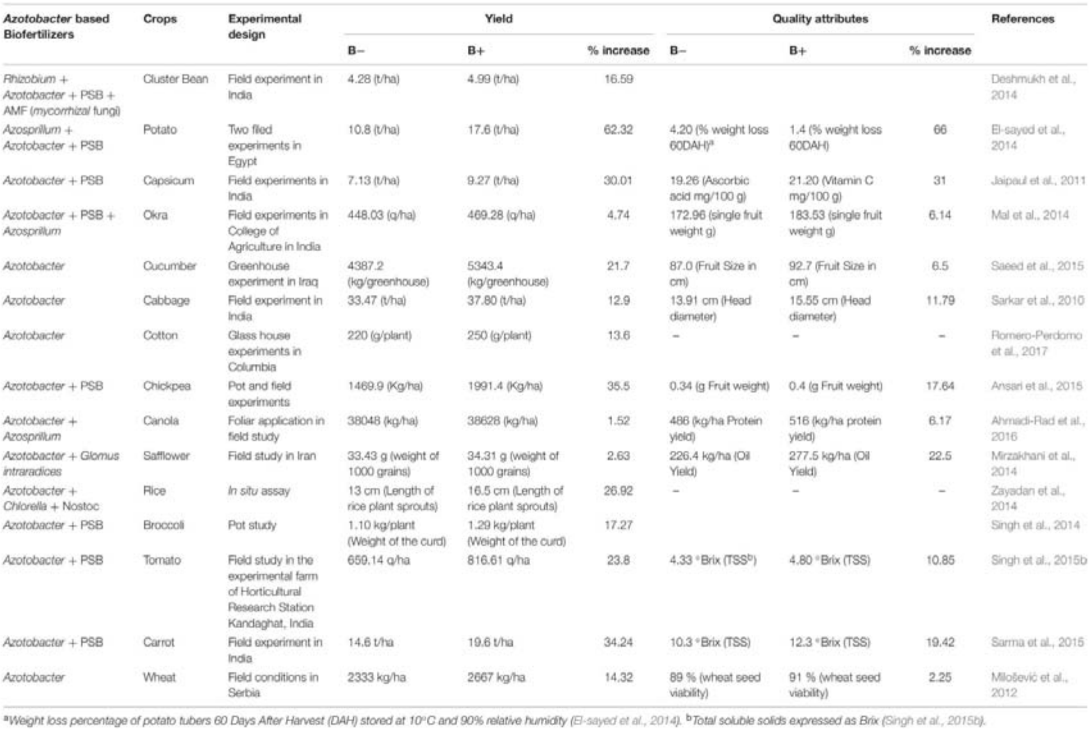
Burkholderia strains often possess multiple plant-growth-promoting (PGP) functions (nitrogen fixation, phosphate solubilization, siderophore production) and have been shown in greenhouse trials to enhance nutrient use efficiency (of both N and P) and aboveground biomass in maize [3].

Burkholderia species are versatile endophytic and rhizosphere colonizers that contribute to plant growth through nitrogen fixation, siderophore production, and ACC deaminase activity, which reduces ethylene stress under adverse conditions. Inoculation with *Burkholderia* strains has been shown to enhance nutrient uptake and biomass production in various crops. Most notably it is related to tree species. “Compared to the mock-inoculated plants, strain S39-2-inoculated plants showed a significantly higher biomass yield, with a 60.4% increase in root biomass and a 44.2% increase in shoot biomass. Strain S39-2 displayed a clear *in planta* nitrogenase activity, which was associated with a 53.5% increase in leaf nitrogen content over control plants.” [6] (Fig. 5).

1.4. Herbaspirillum seropedicae

Herbaspirillum seropedicae is an endophytic bacterium that strongly associates with grasses such as maize, rice, and sugarcane. It colonizes both the rhizosphere and plant tissues, establishing endophytic populations that actively fix nitrogen. “Inoculation with *H. seropedicae* has been shown to increase nitrogen content in plants and improve growth, particularly under nitrogen-limited conditions. Fertilization at 40 kg N ha^{-1} plus bacterial inoculation produced crop yields similar to the treatment with 80 kg N ha^{-1} and increased grain N content, especially in the off-season with 40 kg N ha^{-1}. The BNF contributed about 30% of N accumulated in plants inoculated with ZAE94.” [7].

Herbaspirillum seropedicae has been shown to contribute nearly 25–27% of nitrogen in wheat shoots and grains via N_2 fixation when inoculated, increasing shoot and grain yields by up to 31% in pot experiments [1].



Azotobacter based Biofertilizers	Crops	Experimental design	Yield			Quality attributes			References
			B−	B+	% increase	B−	B+	% increase	
Rhizobium + *Azotobacter* + PSB + AMF (*mycorrhizal* fungi)	Cluster Bean	Field experiment in India	4.28 (t/ha)	4.99 (t/ha)	16.59				Deshmukh et al., 2014
Azosprillum + *Azotobacter* + PSB	Potato	Two filed experiments in Egypt	10.8 (t/ha)	17.6 (t/ha)	62.32	4.20 (% weight loss 60DAH)[a]	1.4 (% weight loss 60DAH)	66	El-sayed et al., 2014
Azotobacter + PSB	Capsicum	Field experiments in India	7.13 (t/ha)	9.27 (t/ha)	30.01	19.26 (Ascorbic acid mg/100 g)	21.20 (Vitamin C mg/100 g)	31	Jaipaul et al., 2011
Azotobacter + PSB + *Azosprillum*	Okra	Field experiments in College of Agriculture in India	448.03 (q/ha)	469.28 (q/ha)	4.74	172.96 (single fruit weight g)	183.53 (single fruit weight g)	6.14	Mal et al., 2014
Azotobacter	Cucumber	Greenhouse experiment in Iraq	4387.2 (kg/greenhouse)	5343.4 (kg/greenhouse)	21.7	87.0 (Fruit Size in cm)	92.7 (Fruit Size in cm)	6.5	Saeed et al., 2015
Azotobacter	Cabbage	Field experiment in India	33.47 (t/ha)	37.80 (t/ha)	12.9	13.91 cm (Head diameter)	15.55 cm (Head diameter)	11.79	Sarkar et al., 2010
Azotobacter	Cotton	Glass house experiments in Columbia	220 (g/plant)	250 (g/plant)	13.6	–	–	–	Romero-Perdomo et al., 2017
Azotobacter + PSB	Chickpea	Pot and field experiments	1469.9 (Kg/ha)	1991.4 (Kg/ha)	35.5	0.34 (g Fruit weight)	0.4 (g Fruit weight)	17.64	Ansari et al., 2015
Azotobacter + *Azosprillum*	Canola	Foliar application in field study	38048 (kg/ha)	38628 (kg/ha)	1.52	486 (kg/ha Protein yield)	516 (kg/ha protein yield)	6.17	Ahmadi-Rad et al., 2016
Azotobacter + *Glomus intraradices*	Safflower	Field study in Iran	33.43 g (weight of 1000 grains)	34.31 g (weight of 1000 grains)	2.63	226.4 kg/ha (Oil Yield)	277.5 kg/ha (Oil Yield)	22.5	Mirzakhani et al., 2014
Azotobacter + *Chlorella* + Nostoc	Rice	*In situ* assay	13 cm (Length of rice plant sprouts)	16.5 cm (Length of rice plant sprouts)	26.92	–	–	–	Zayadan et al., 2014
Azotobacter + PSB	Broccoli	Pot study	1.10 kg/plant (Weight of the curd)	1.29 kg/plant (Weight of the curd)	17.27				Singh et al., 2014
Azotobacter + PSB	Tomato	Field study in the experimental farm of Horticultural Research Station Kandaghat, India	659.14 q/ha	816.61 q/ha	23.8	4.33 °Brix (TSS[b])	4.80 °Brix (TSS)	10.85	Singh et al., 2015b
Azotobacter + PSB	Carrot	Field experiment in India	14.6 t/ha	19.6 t/ha	34.24	10.3 °Brix (TSS)	12.3 °Brix (TSS)	19.42	Sarma et al., 2015
Azotobacter	Wheat	Field conditions in Serbia	2333 kg/ha	2667 kg/ha	14.32	89 % (wheat seed viability)	91 % (wheat seed viability)	2.25	Milošević et al., 2012

[a] *Weight loss percentage of potato tubers 60 Days After Harvest (DAH) stored at 10°C and 90% relative humidity (El-sayed et al., 2014).* [b] *Total soluble solids expressed as Brix (Singh et al., 2015b).*

Source: [2].

Fig. 4. Effect of *Azotobacter* based biofertilizers on yields and quality improvement of different crops

1.5. Pseudomonas sp.

While traditionally recognized for biocontrol, certain *Pseudomonas* strains also contribute to nitrogen fixation. They produce antibiotics, siderophores, and phytohormones, providing both growth promotion and protection against soil-borne pathogens. Inoculation with *Pseudomonas* strains has been shown to enhance plant growth and yield, particularly in integrated nutrient and pest management systems [9].

In potatoes Biweekly applications of LBUM223 significantly increased potato tuber yield by 46% (P=0.048) compared with control plots. Results for the treatment with a single application with LBUM223 were more variable, even within the same experimental year, and therefore did not significantly differ from the control plots or the ones having received biweekly inoculations with LBUM223 [10] (Fig. 6).

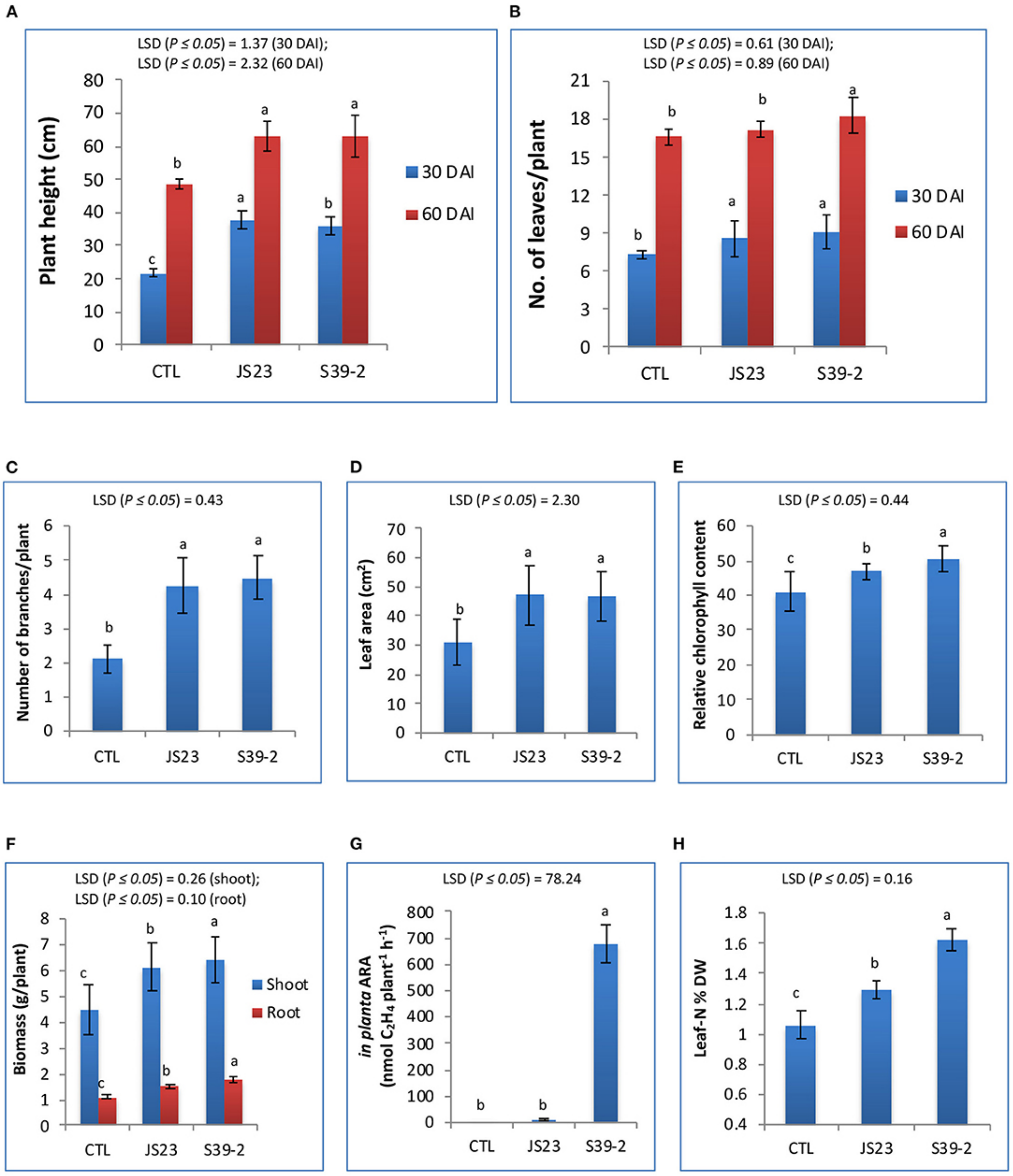
1.6. Bacillus sp.

Bacillus species are robust spore-formers, making them resilient to environmental stresses and suitable for long-term storage as inoculants. While their nitrogen contribution is less studied, they promote growth through phytohormone production, nutrient solubilization, and biocontrol via lipopeptides and induced systemic resistance (ISR). Field applications often use spore-based formulations for seed treatment, soil amendment, or foliar sprays, offering both nutrient and disease management benefits [11].

1.7. Other diazotrophic bacteria

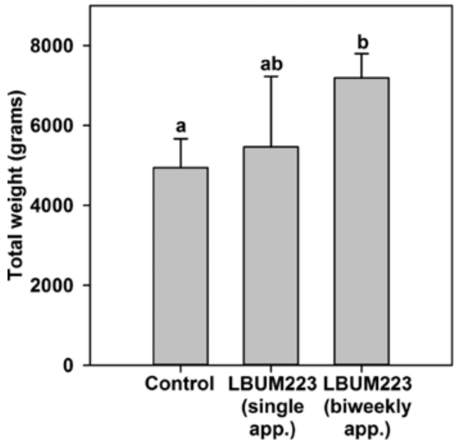
Science is constantly evolving, and new diazotrophic bacteria continue to be discovered that are not discussed in this article.

Iron-reducing bacteria, Anaeromyxobacter and Geobacter, are newly found diazotrophic bacteria predominant in paddy soil. Experimental field of this study is a long-term (35 years) nitrogen fertilized (6.0 g N/m2/year) and unfertilized paddy field, where ca. 70% of rice yield was obtained yearly in nitrogen unfertilized plot (443 ± 37 g/m^2) compared to fertilized plot (642 ± 64 g/m^2). Effects of long-term nitrogen fertilization/unfertilization on soil properties related to BNF were investigated with special reference to diazotrophic iron-reducing bacteria. It was concluded that long-term use/unuse of nitrogen fertilizer in this field did not affect the predominance and nitrogen-fixing activity of diazotrophic iron-reducing bacteria, composition of other general diazotrophs, and the resulting soil nitrogen-fixing activity. BNF, primarily driven by diazotrophic iron-reducing bacteria, might significantly contribute to sustain soil nitrogen fertility and rice yield in both plot soils [13].



Source: [6].

Fig. 5. PGP effects of non-diazotrophic and diazotrophic isolates on eucalyptus seedlings



Source: [10].

Fig. 6. Total weight of tubers at harvest (week 17). Pseudomonas fluorescens LBUM223 was inoculated either once at planting (single app.) or at biweekly intervals (biweekly app.)

1.8. Gene editing for higher performance of diazotrophic bacteria

Gene editing can be used to enhance the performance of diazotrophic bacteria by improving their nitrogen-fixing efficiency, stress tolerance, and compatibility with host plants. These genetic modifications aim to boost crop productivity and reduce reliance on synthetic nitrogen fertilizers.

Long-term ecological studies in legume–rhizobia interactions have shown that elevated nitrogen inputs can lead to the evolution of less cooperative nitrogen-fixing mutualists. Here we describe how reprogramming the genetic regulation of nitrogen fixation and assimilation in a novel root-associated diazotroph can restore ammonium production in the presence of exogenous nitrogen inputs. We isolated a strain of the plant-associated proteobacterium Kosakonia sacchari from corn roots, characterized its nitrogen regulatory network, and targeted key nodes for gene editing to optimize nitrogen fixation in corn. While the wild-type strain exhibits repression of nitrogen fixation in conditions replete with bioavailable nitrogen, such as fertilized greenhouse and field experiments, remodeled strains show elevated levels in the rhizosphere of corn in the greenhouse and field even in the presence of exogenous nitrogen. Such strains could be used in commercial applications to supply fixed nitrogen to cereal crops [14].

We demonstrate that R. sp. IRBG74 can be engineered to result in nitrogenase activity under free-living conditions by transferring a nif cluster from either Rhodobacter sphaeroides or Klebsiella oxytoca. For P. protegens Pf-5, the transfer of an inducible cluster from Pseudomonas stutzeri and Azotobacter vinelandii yields ammonium tolerance and higher oxygen tolerance of nitrogenase activity than that from K. oxytoca. Collectively, the data from the transfer of 12 nif gene clusters between 15 diverse species (including Escherichia coli and 12 rhizobia) help identify the barriers that must be overcome to engineer a bacterium to deliver a high nitrogen flux to a cereal crop [16].

2. Potential for quantifiable nitrogen fixation.

2.1. Many papers demonstrate positive results

The biological fixation of atmospheric N_2 is an essential process in the biosphere, second in importance only to photosynthesis for the maintenance of life on earth [21]. Only a few key genera of prokaryotic organisms that contain the genetic information needed to synthesize the enzyme nitrogenase possess the ability to convert gaseous N_2 into NH_3 which can then be biochemically modified to generate different organic forms of N [12].

“Despite the adoption of relatively good management practices for synthetic N by many cereal-growers, N use efficiency (NUE) (measured as kg plant N harvested per kg synthetic fertilizer N applied) is frequently less than 50% [12]. This inefficiency in the supply of artificial nitrogen fertilizers contrasts sharply with the more synchronized release and uptake of organic nitrogen sources. In essence, organic nitrogen can be considered roughly twice as efficient as synthetic fertilizer nitrogen in supporting crop growth.

Different methodologies have been employed in the study of biological nitrogen fixation (BNF) in both free-living and plant-associated N_2-fixing systems. As a result, the data are often heterogeneous, highlighting the need for simplification and standardization to enable meaningful comparisons and draw practical conclusions for farm-level applications.

Some data come from a mix of controlled environment and field investigations using multiple measurement techniques, such as the recent report that 29–82% of N acquired by a Mexican indigenous maize landrace grown on unfertilized N-deficient soil over five years appeared to be derived from BNF [22]. Based on the estimates of %Ndfa and the amounts of N accumulated by crops it was calculated that inputs of BNF could have represented up to 122 kg N ha^{-1} yr^{-1}, although at most locations determinations were within the range of 4–15 kg N ha^{-1} yr^{-1} [22].

Most inoculation experiments include uninoculated treatments as “non-fixing” references, while in 15N-based studies comparing crop germplasm either the cultivar exhibiting the lowest 15N excess or the measured 15N excess of soil available N have been used as reference controls. While estimates of extraordinary high %Ndfa values have been reported (highest values for rice 59%, maize 82% and wheat 85%, most %Ndfa determinations for inoculation or cultivar comparisons have been < 33% across all three crops [12].

In flooded conditions under favourable conditions, cyanobacteria can fix 20–40 kg N ha^{-1} $crop^{-1}$ [23].

Several studies in rice where N balance calculations were constructed taking all known N inputs into account, have indicated positive N balances ranging from 18 to 51 kg N ha^{-1} $crop^{-1}$. There have been fewer similar undertakings for other cereals, but net N balances have been reported from 13 to 35 kg N ha^{-1} $crop^{-1}$ for wheat and 13–26 kg N ha^{-1} $crop^{-1}$ for maize [12].

15N2-labeling field-based growth chamber studies have been undertaken in China [24–28] which included measurements of BNF by rice plants grown close to maturity and those estimates of non-symbiotic N_2 fixation ranged from 19 to 51 kg of N ha^{-1} $crop^{-1}$. One of these studies [25] calculated 23 and 39 kg of fixed N ha^{-1} for the whole plant-soil systems with inbred japonica (W23) and hybrid indica (IIY) rice cultivars, respectively, but only 1–2.5% of this fixed N was detected in rice plants or weeds. This was consistent with earlier conclusions that much of the non-symbiotic fixed N_2 enters via SON rather than directly supporting the nutrition of the current crop. It is important to note that except for the study by Zhang et al. [28] all other investigations were carried out in soil without application of synthetic N, which is atypical of most rice production systems. Zhang et al. [28] reported an 81–86% reduction in rates of N_2 fixation when fertilizer N was applied at rates of 125–250 kg N ha^{-1}, yet nifH copy number increased. Similar 15N2 studies are also needed for wheat and maize under common farmer N fertilization practices [12].

Numerous short- and long-term research trials carried out globally over the last five decades suggested that on an average, wheat, rice and maize obtained 48% of their N from fertilizer and 52% of N from soil sources [29. If the native soil organic matter provided the bulk of this N, the soil N reserves would be expected to be progressively depleted over time [31, 32]. Yet a meta-analysis of measured changes in SON in 114 long-term continuous cereal experiments conducted globally did not indicate the anticipated extent of decline [30]. Instead, the soils cultivated with cereals seemed to have approached a more-or-less steady state, suggesting that in addition to fertilizer N, there were other sources of N inputs provided to cereal-based cropping systems that contributed to replenishing much of the soil N either lost or removed in harvested products. Such other sources of N could include inputs of N via (a) farm-yard manure, (b) the planted seed, (c) recycling of N from above-ground crop residues, (d) atmospheric N deposition (via both rain and dust), and (e) non-symbiotic BNF in soil and plant systems. After considering the estimates of contributions of N from all other potential sources, the unexplained balance representing 24.6% of N removed by cereals was attributed to non-symbiotic BNF [29]. These calculations implied that BNF played a key role in maintaining the observed N equilibrium in the soil N pool under continuous cereal cultivation and constant crop management. (12).

While a theoretical upper limit of 60–80 kg N ha^{-1} $crop^{-1}$ has been proposed for non-symbiotic N_2 fixation in cereals by assuming there is an abundant population of diazotrophs in and around the plant and unlimited pools of available C, such high values are unlikely to be attainable. The BNF potentials listed for aquatic green manures (260 kg N ha^{-1} $crop^{-1}$) and grain legumes (245–290 kg N ha^{-1} $crop^{-1}$; Table 1) on the other hand are not unprecedented or unrealistic, as comparable values have already been reported in the literature [12].

Commonly reported range in estimates of BNF (kg N ha^{-1} $crop^{-1}$), proposed potential levels achievable, and the prospective outlook for improving BNF inputs from various N2-fixing systems (Table 1).

2.2. Some studies argue that diazotrophic bacteria do not fix agronomically significant amounts of atmospheric dinitrogen (N_2)

While it is widely accepted that there is a diverse range of bacteria that exist as free-living nitrogen fixers in soils and as plant endophytes and epiphytes, there is some debate as to whether or not they contribute agronomically significant amounts of fixed nitrogen to resident plants [17].

We no find unequivocal evidence that these bacteria fix agriculturally significant amounts of N2 from the atmosphere in non-legumes. Research since the 1930s has followed repeated, overlapping cycles that have concluded that plant-growth-promoting hormones were the primary reason for crop response to microbial inoculants [15].

However, these studies do not dispute the plant growth–promoting effects observed; rather, they attribute them to mechanisms other than biological nitrogen fixation (BNF).

Table 1. BNF potential

BNF system	Common range of reported BNF	Theoretical maximum BNF potential		Advantage	Constraint	Status of adoption by farmers	Outlook
		Proposed	Assumptions				
Total non-symbiotic N_2 fixation	rice: 18–51	60–80 in rice, wheat, and maize	Prolific populations of endophytic and rhizospheric N_2-fixing bacteria	Inherent to the system	Prone to N loss	Widely used by default	Potential to improve through agronomic (including straw) management as part of soil health agenda
	wheat: 3–40 maize: 13–26		All C input (2 t crop^{-1}) is used by N_2 fixers		Improvement is difficult		
			40 mg N is fixed g C^{-1}				
Cyanobacteria in rice cultivation	0–80	70	Photosynthetic aquatic biomass is composed of exclusively of N_2-fixing BGA (C:N = 7)	Inherent to the system	Requires continuous standing water	Widely used by default	Low potential because of difficulty in managing the algal bloom as inoculations do not work
			Primary production is 0.5 t C ha^{-1} crop^{-1}		Inhibited by combine N in flood water		
					Grazer inhibits growth of cyanobacteria		
***Azolla* in rice cultivation**	20–150	225	Two *Azolla* crops grown and incorporated per rice crop	High (>80%) %Ndfa and large amounts of N produced	Requires continuous standing water on soil surface	Use by the farmers has declined, and currently negligible	Low or negligible potential
				Improves SOM	Labor intensive		
				Reduces N volatilization loss	Difficult in maintaining inoculum supply		
				Reduces weed pressure			
Aquatic legume green manure in rice cultivation	20–260	260 in 55 days	Fast-growing species such as *Sesbania rostrata* is used as green manure	High %Ndfa (80–90%) and large amount of N production	Farmers prefer legumes with economic value	Use by the farmers declined, currently insignificant use	Modest potential in single rice cropping system in Africa and some parts of Asia
				Improves soil organic matter	Labour		
					Intensive		
Grain legumes in cereal rotations	57 kg total N fixed ha^{-1} (common bean) to 212 kg total N fixed ha^{-1} (faba bean)b	245–290 kg total N fixed ha^{-1}	Legume crops other than common bean	Inherent to the system	Dominant cereals restrict legume cultivation	Widely adopted by the farmers, but their inclusion in farming systems driven by fluctuations	High potential to enhance yield and improve consistency of legume productivity through agronomic management
			10–12 t shoot dry matter ha^{-1} (3.5–4 t grain ha^{-1})	Provide multiple rotational benefits that improve ce-	Cereals have larger markets and are easier to grow than legumes		

				real productivity		in market demand and value	and breeding
			%Ndfa of 85%, 20 kg N fixed per t shoot dry matter accumulated		Grain economic value is highly volatile		
			Nodulated roots represent 30% of total crop N		Many pulses are susceptible to disease and insect pests		

Note: Adapted from [12]

3. Practical approach to implementation and experimentation with PGPR

Since direct measurement of nitrogen fixation efficiency from plant growth-promoting rhizobacteria (PGPR) is difficult to assess due to the complex mechanisms governing nitrogen supply to both plants and soil, reported values vary widely among studies—from 3 to 80 kg N ha^{-1} $crop^{-1}$ under non-flooded conditions, and up to 260 kg N ha^{-1} $crop^{-1}$ in flooded or aquatic systems.

The difference between flooded and not flooded fields can be attributed to the presence or lack of oxygen.

Oxygen irreversibly inhibits nitrogenase, even in aerobic organisms. Therefore, diazotrophs must employ protection mechanisms to maintain N fixation when oxygen is present [18].

Clearly, farmers cannot rely on such broad ranges for practical implementation on their farms. The heterogeneity in these data is most likely influenced by multiple interacting parameters affecting the performance and efficiency of free-living nitrogen-fixing bacteria. These include the quantity of soil organic matter and organic carbon, soil physical and chemical properties, the specific plant–soil–microbe combination, and the type and rate of nitrogen fertilizer applied. Given this multitude of variables, significant variability in results across farms is to be expected. Therefore, farmers are advised to conduct small-scale field trials using PGPR inoculants to identify the optimal balance between reduced nitrogen inputs, bacterial strain (or strain combinations), and inoculation rates that achieve sustainable yields with lower input costs or improved productivity at reasonable expense.

While many studies have established that non-symbiotic N_2 fixation occurs in the soil-plant habitat, most of them do not represent actual field settings, for multiple reasons. Trials were often undertaken at small scale, sometimes crops were also not grown to maturity, and experimental constraints may have resulted in sub-optimal plant growth conditions [12].

However it is known that some agronomic practices can increase BNF efficiency. Especially practices increasing Soil Organic Carbon (SOC).

The addition of plant- and microbial-derived C significantly enhanced soil nitrogenase activity (13~28%) and that microbial-derived C had a more positive impact. These positive effects were attributed to the direct C-energy supply (0.49~0.84) rather than variations in soil microbial activity (-0.01~0.21) and substrate resources (-0.45~0.27). Long-term N addition did not inhibit FLNF. C addition promoted FLNF in soils of the two forests, but the response rate was higher in the leguminous forest soils. Our study reveals that increased soil C availability can drive FLNF in tropical forests, enhancing our understanding of the soil C-N coupling mechanism [19] (Table 2).

Table 2. Agronomic practices with potential to enhance BNF inputs in cereal-based farming systems [12]

Ractice	Likely mechanism for enhancement of BNF inputs	References
Zero or reduced tillage	Positive changes in diversity and heterogeneity of rhizosphere diazotrohphic community	Li et al. (2021)
	Higher organic matter and substrate inputs in rhizosphere	Zhou et al. (2020)
	Lower soil nitrate from reduced disturbance of soil organic matter reduces risk of inhibition of BNF	Peoples et al., (1995), Torabian et al. (2019)
Crop residue retention	Availability of a wide range of C compounds as source of C and energy substrates by diazotrophs	Roper and Ladha (1995)
	Residue mulch creates conducive microenvironment (i.e., moisture conservation, lower O_2 environment, steady supply of C) for diazotrophs	Fan et al. (2020)
	Crop residue of high C:N (i.e., cereal straw) immobilizes inorganic N result in stimulation of BNF	Palm et al. (2014)

Smart synthetic N management	Optimal rate and timely application of synthetic N to cereals improves N use efficiency and reduces risk of unutilized fertilizer N inhibiting BNF by diazotrophs during cereal phase	Ladha et al. (1998)
Application of biochar	Biochar enriches soil and stores organic C in a form that provides C and energy source for diazotrophs	Laird (2008)
	Biochar immobilizes inorganic N so BNF less likely to be suppressed	Nelson et al. (2011)
	Increases P bioavailability which stimulates BNF	Thies and Rilling (2009)
Use of manure with or without inorganic fertilizer.	Enhances soil C storage and nutrient availability after decomposition which will serve as C and energy source for diazotrophs	Ladha et al. (2011)
	Supports more diverse soil microbial communities and increases microbial biomass contributing to increase in BNF	
Increased water availability	Drought suppresses BNF process	Peoples et al., (1995), Santachiara et al. (2019)
Controlled water application	Adequate plant-available water via rainfall or irrigation increases BNF by stimulating plant growth and microbial activity	
	'Saturated soil culture' (long-term flooding) enhances nodulation and BNF by soybean	
Integration of legume in fallow or in rotation as part of diversification and intensification	Increased frequency of use of legumes in cropping system results in increased inputs of BNF	Franke et al. (2018)
	Supply of in situ high quality residues with high N concentration and a low C:N ratio improves soil N status	
Green or brown legume manurea	Increased frequency of use of legumes in cropping system results in increased inputs of BNF	Becker et al. (1995), Singh et al. (2009)
	Supply of in situ legume residue with high N concentration and a low C:N ratio improves soil N status	Peoples et al. (2017)
	Green manure mulch and brown manuring assist the management of weeds	
Intercropping legumes within cereals	Increased frequency of use of legumes in cropping system results in increased inputs of BNF	Lithourgidis et al. (2011)
	Intercropped legume has higher %Ndfa than legume sole crop	Bedoussac et al. (2015)
	Increased yield stability and yield per unit area, reduced pest problems and lower requirements for agrochemicals and N fertilizer to support cereal yield	Fletcher et al. (2016)
		Jensen et al. (2020)

There is another potential uncertainty regarding the viability of the inoculum.

Although numerous bacterial and cyanobacterial inoculation trials have been conducted, and inoculants have been commercialized in many countries [33, 34], as far as we are aware few studies have observed consistent results or conclusively demonstrated the successful manipulation of non-symbiotic N_2 fixation inputs in cereals with conventional inoculation technologies under field conditions [35, 36]. Experience with rhizobial inoculants for legumes has demonstrated that a range of diverse factors (i.e., edaphic, biotic, climatic) can limit the effectiveness of inoculation, but that poor inoculum production, storage and/or application practices can also be responsible for many inoculation failures and inconsistent results [37–39]. The same challenges face the establishment and survival of sufficient populations of any new inoculant diazotroph species or strains within the existing soil microbial community that might be necessary to elicit an inoculation response.(12)

4. Conclusion

Based on the reviewed literature, the following recommendations are proposed for farmers considering the implementation of plant growth-promoting rhizobacteria (PGPR) to reduce nitrogen fertilizer costs:

- Set realistic expectations.
- PGPR inoculation should not be expected to deliver miraculous results. A reasonable expectation for an outcome is either a 5–20% yield increase at the current fertilizer application rate, or the possibility to reduce nitrogen fertilization by up to 20% while maintaining the same yield. Under specific favourable conditions, higher responses may occur.
- Identify conditions favourable to PGPR activity.

- Free-living PGPR bacteria perform best in unfertilized fields, soils with disturbed or depleted biological activity, and soils with high organic carbon (SOC). In other words, they tend to show greater effectiveness under extensive rather than intensive management systems.
- As noted, “Normally, heterotrophic free-living diazotrophs are active, fixing N_2 in surroundings rich in organic C and low in N.” [12].
- Do not rush into full-scale application of PGPR or discontinue nitrogen fertilization entirely.
- At present, the author has not encountered consistent evidence showing that crops inoculated with free-living nitrogen-fixing PGPR achieve higher yields than well-fertilized control treatments. However, there is strong and consistent evidence supporting a reasonable 5–20% reduction in nitrogen fertilization when substituted with nitrogen-fixing PGPR inoculation, without compromising yield performance.
- Recognize the importance of soil–plant–microbe compatibility.
- The efficiency of PGPR depends on the specific interaction between the soil, crop species, and bacterial strain. Farmers are therefore encouraged to establish small test plots using different strains or strain combinations to identify the most effective inoculant for their conditions. Because each field has unique characteristics, the optimal solution cannot be prescribed universally by agronomists or product vendors—on-farm trials are essential before scaling up.

Suggested test plot design for evaluating PGPR inoculation efficiency under farm conditions (Table 3).

Table 3. Suggested on-farm test plot design prior to large-scale implementation of PGPRB treatment

Plot No.	Nitrogen fertilization level	PGPR inoculation	Purpose/Notes
1	None	None	Absolute control — baseline for natural soil fertility.
2	Regular (current farmer’s rate)	None	Standard management — yield reference for comparison.
3	None	Applied	Tests effect of PGPR alone without N input.
4	20% lower than regular	None	Measures yield response to reduced N without PGPR.
5	20% lower than regular	Applied	Tests PGPR’s ability to compensate for modest N reduction.
6	50% lower than regular	None	Evaluates the threshold of N limitation without PGPR.
7	50% lower than regular	Applied	Tests PGPR efficiency at strong N reduction; identifies potential for substitution.

Different PGPR strains and strain combinations should each be tested in separate sets of the above treatments to determine the most effective inoculant for local soil and crop conditions.

The literature indicates that the greatest percentage increases occur in extensive, non-fertilized fields; however, these systems still fail to reach the high yields achieved in well-fertilized fields. An economic evaluation is therefore required to determine whether an extensive system could be more profitable than an intensive one.

This plot design will generate sufficient data not only to evaluate the effectiveness of PGPR inoculation, but also to assess the efficiency of current nitrogen fertilization practices, which in many cases exceed economically justified levels.

Consider the broader potential of PGPR.

The benefits of PGPR extend beyond nitrogen fixation alone. They can contribute to disease suppression, enhanced stress tolerance, phytohormone production, and improved nutrient mobilization, offering a holistic approach to sustainable crop productivity.

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СВОБОДНО ЖИВЕЕЩИ АЗОТОФИКСИРАЩИ РАЗТЕЖ-ПОДПОМАГАЩИ РИЗОБАКТЕРИИ: ЕКОЛОГИЧНИ РОЛИ И ПРИЛОЖЕНИЯ

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Резюме

Корените на растенията са обитавани от голямо разнообразие от микроорганизми, някои от които са полезни за растежа на растенията и са известни като ризобактерии, стимулиращи растежа на растенията (PGPR). Свободно живеещите и асоциативните азотфиксиращи растежа на растенията стимулиращи ризобактерии (PGPR) представляват обещаваща устойчива алтернатива на химическите торове, като осигуряват биологично фиксиран азот (N_2) и множество дейности, стимулиращи растежа на растенията. Видовете *Azospirillum*, *Azotobacter*, *Herbaspirillum seropedicae*, *Burkholderia*, *Pseudomonas* и *Bacillus* са показали способността да фиксират азот при несимбиотични или слабо симбиотични отношения с нелегуминозни култури, да произвеждат фитохормони, да разтварят фосфор, да потискат патогени и да подобряват устойчивостта към стрес. Чрез тези многопосочни взаимодействия, PGPR не само подобряват усвояването на хранителни вещества, но и укрепват защитата на растенията, допринасяйки за по-здрави и по-устойчиви култури. Въпреки многобройните обнадеждаващи открития, все още съществуват предизвикателства при точното определяне на ефективността на фиксацията на азот поради огромната сложност на взаимодействията в почвената биома и разнообразните механизми, чрез които бактериите влияят на физиологията на растенията. Целта на това проучване е да се представи практически сравнителен анализ, който да помогне на фермерите да разберат как могат ефективно да намалят употребата на химически торове чрез прилагане на PGPR инокулации.

Ключови думи: *Rhizobacteria*, азотофиксиране, PGRP.