Biological Nitrogen Fixation in Agriculture

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Biological nitrogen fixation (BNF) is a natural process of changing atmospheric nitrogen (N_2) into a simple soluble nontoxic form (NH_4+ primarily) which is used by plant cell for synthesis of various biomolecules. Nitrogen fixation is one of the major sources of nitrogen for plants and a key step distributing this nutrient in the ecosystem. Optimization of BNF is critical to sustain both food production and environmental health.

Keywords: BNF ; biofertilizers ; legumes ; yield improvement

1. Inoculants for Legume Crops

N input through BNF is approximately 122 million tons of *N* per year of which 55 to 60 million tons is fixed by agricultural crops [1][2][3]. Soybean (*Glycine max*) legume has the highest contribution of BNF; this species fixes annually ~16.4 million tons of *N* [4]. The main microsymbionts of soybean belong to *Bradyrhizobium* species [5].

The potential of BNF providing nitrogen *N* in ecosystems is increasingly being exploited in agricultural practices, mostly through legume cultivation (Soybean, lupin, alfalfa, chickpea, cowpea, etc.). Legume–rhizobium symbiosis is an important facet of symbiotic nitrogen fixation [6][7]. Inoculation of legumes crops with Rhizobia is one of the success stories of biofertilizers in agriculture. The positive impact of diazotrophic microorganisms on agriculture has opened the biofertilizer market. In few years the biofertilizer market has grown and at present, many nitrogen-fixing microorganisms are marketed as biofertilizers (**Table 1**). Different products are available and some of them have shown great potential by improving crop growth and yield and could significantly reduce a farmer's fertilizer bill (**Table 1**). For example, in Brazil, the economic benefit in terms of *N*-fertilizer saving was over USDA 2.5 billion per year by 2002 [8]. Use of BNF-based commercial inoculums has contributed to increase soybean yield in Brazil, and therefore helped to put the country in second place among the largest soybean producers behind the USA. In the USA, the contribution of BNF to the soybean *N* nutrition ranged from 23 to 65% [9]. In Spain, Pastor-Bueis et al. [10] showed that *Rhizobium leguminosarum* bv. *phaseoli* LCS0306, formulated with perlite-biochar carriers, produced a significantly higher grain yield of common bean (3640 kg ha^{-1} versus 3165 kg ha^{-1} in the *N*-fertilized control plot). In Ghana, Ulzen et al. [11], by comparing urea application to two commercial biofertilizers (Biofix and Legumefix) on soybean and cowpea, reported that these inoculants were more profitable. They increase nodule dry weight (>two-fold), nodule number (90–118%), and grain yield (12–19%) compare to the control (urea). In northern Nigeria, Ronner et al. [12] also showed that soybean inoculation with rhizobia has increased yield by 447 kg/ha compared to the control. Similar results were reported by Thuita et al. [13] who recommended for sustainable soybean yield increase, to inoculate with Legumefix + sympal (a fertilizer blend for use with rhizobia inoculants) or biofix + sympal to raise yields from 2000 kg/ha to 4000 kg/ha. In poor soils, amendment with vermicompost in addition to Sympal and Legumefix has been shown to improve soybean yields [14]. Previously, in a study of several commercial rhizobial inoculum, Thuita et al. [15] reported that these products have potential to increase growth, yield, and nitrogen fixation legumes. A noteworthy contribution of the use of legume inoculants was also reported in the Zambian's economy, with an input of more than US $23 million in eight years [16]. Recently "Nitragin" a pure culture of root-associated bacteria was improved and tested on soybeans and soyfoods in Germany. Results showed that soil inoculated with Nitragin gave a 3- to 4-fold increase in yield, plus an increase in protein in the roots and leaves [17].

Table 1. Some famous marketed microbe-based biofertilizers and target crops.

Name of Manufactured Products and Producer (in Italic)	Strain	Formulation	Crops Suited	Benefits According to the Authors	References
BioGro *Nguyen Thanh Hien in Hanoi University (Vietnam)*	*Pseudomonas fluorescens* *Bacillus subtilis* *Bacillus amyloliquefaciens* *Candida tropicalis*	Solid in peat	Rice (*Oryza sativa*)	Improve rice yield	[18]
Biofix *MEA company limited (Kenya)*	Rhizobium	solid	-Soya bean (*Glycine max*) -Common bean (*Phaseolus vulgaris L*) -Alfalfa (*Medicago sativa)*	Cheaper than chemical nitrogen Lighter to transport, requires less labor effective for many crops	[19]
Bio N *Nutri-Tech Solutions* (*Australia*)	*Azotobacter* spp.	liquid	Horticulture	Access free atmospheric nitrogen. Increase yield and quality. Reduce soil erosion. Improve water retention. Enhance germination. Promote root growth. Phosphate release	[20]
Microbin and Azottein *Egyptian Ministry of Agriculture*	*Klebsiella*, *Bacillus*, *Azotobacter* *Azospirillum*	Carrier material	Barley cultivar Giza	Increased the different plant characteristic increases in grain yield reached approximately 24.8 and 27.2%	[7][21]
Legumefix *Legume Technology (UK)*	*Bradyrhizobium japonicum*	Sterile peat inoculant	Soybean and cowpea	grain yield (12–19%) relative to the control	[11]
Leguspirflo *SoyGro (South Africa)*	*Azospirillum brasilense*	Liquid	soybean	Inefficient	[12]
TerraMax's Micro AZ product *TerraMax (Minnesota, USA)*	*Azospirillum brasilense* *A. lipoferum.*	Liquid	Wheat, Corn and Grain Sorghum	Improve root structure and stimulate root growth Provide biological nitrogen fixation Increases yields Stimulates rooting Increases yields	[22][23]
Nitrofix P *Agro-Input Suppliers Limited (AISL) (Malawi)*	*Bradyrhizobium japonicum* and *Bradyrhizobium elkanii*	Dry- inoculum based on gamma-sterilized peat	Soybeans	Promotes an increase in the yield by an average of 14.3–20.3% Reduced the nitrogen requirement	[24]
Vault LVL *BASF (Badische Anilin- & Soda-Fabrik) Germany*	*B. japonicum* + *Bacillus subtilis*	Liquid	Soybeans	Biomass yield improved	[13][15]

2. Inoculants for Non-Legume Crops

Several non-leguminous plants, mainly cereals, have developed multiple strategies in association with diazotrophs to cope with *N* deficiency. Some of these microorganisms have been used to make bacterial inoculants. Mexico was one of the first countries to commercialize maize seeds coated with *Azospirillum* [25], followed by Argentina. Field experiments in Sierra Mixe (region of Oaxaca, Mexico) using ^{15}N natural abundance or ^{15}N-enrichment assessments over 5 years indicated that atmospheric nitrogen fixation contributed to 29–82% of the nitrogen nutrition of maize [26]. In Egypt, El-Sayed et al. [21] showed significant increases (24.8 and 27.2% in the first season and 18.4 and 22.0% in the second season respectively compared to the un-inoculation) in grain yield of barley after inoculation with biofertilizers (Microbin and Azottein, constituted of a mixture of *P*-dissolving and *N*-fixing bacteria), and these results were comparable to those obtained with chemical fertilizers. More recently, Rose et al. [27] demonstrated that a commercial biofertilizer product known as “BioGro” (**Table 1**) can replace 23 to 52% of *N* chemical fertilizers without loss of yield in rice systems, in Southeast Asia. BNF contribute ~30 kg *N* ha^{-1} per year to rice systems [28]. According to Serna-Cock et al. [29], applying *Azospirillum brasilense, Azotobacter chroccocum,* and *Trichoderma lignorum* as biofertilizer in sugarcane plants (variety CC 934418) can replaces 60% of the nitrogen needed by this cultivar. Corroborating these results, Antunes et al. [30] showed that inoculation with *Herbaspirillum seropedicae*, *Pseudomonas* sp., and *Bacillus megaterium* increase sugarcane (variety RB92579) yield from 18% to 57.31%. Although cereals benefit significantly from diazotrophs, most microbes are unlikely to fix nitrogen in the presence of high rate of chemical fertilizers.

3. Success-Limiting Factors of BNF Application in Agriculture

The BNFs have the capacity to reduce the use of nitrogen fertilizers to ~0.160 billion tons per year, which corresponds to a reduction of 0.270 billion tons of coal consumed in the production process [19]. All these results show that BNF is directly proportional to agricultural sustainability. Despite the advantages of microbial inoculant technology, there still exist some success-limiting factors against a universal utilization. In fact, the efficiency of microbe-based biofertilizers depends on many factors including the targeted crop, edaphic (pH, salinity, and soil type), biotic (competition between introduced and indigenous strains, microbial parasites and predators), and climatic factors [31][32] that can make commercial inoculum counter-productive. Besides competition among microbial strains for resources and plant nodulation, partner fidelity and specificity mediated by genetic and molecular mechanisms are among the success-limiting factors against a universal utilization of microbial inoculants [33][34]. On the other hand, commercial inoculants were often made with one or at most two trains, while under field conditions, plants are associated with many strains which provide them diverse benefits through functional complementarity. Nevertheless, the poor performance of biofertilizers is primarily linked to inappropriate strains and inefficient production technology. Herrmann et al. [20], studying the microbial quality of 65 commercial inoculants manufactured in seven different countries, showed that only 36% of the products could be considered as “pure”. Among the remaining 64% some contained one or several strains of contaminants and some products did not contain any strains. However, the study does not specify the origin of this problem. Is it a loss of viability during the storage time or the quality of the product delivered by the manufacturer? Similarly, In India, the evaluation of the quality of legume inoculants showed that most of the products tested did not contain the optimal amount of rhizobium ($< 10^8$ rhizobia/g of inoculant) and were contaminated by a large amount of non-rhizobial organisms [35]. Therefore, it is a big challenge to maintain viability and purity of microbes in microbial inoculant [36]. In many regions across the world, farmers are not yet familiar with this type of fertilizer which is sensitive to temperature, humidity, time, and storage conditions; that is why they are sometimes confused about quality and expiry dates of biofertilizers [37]. In Africa, the International Institute of Tropical Agriculture (IITA) has been working with regulatory authorities for biofertilizers in Kenya, Uganda, Tanzania, Ethiopia, Nigeria, and Ghana to establish standards for both registration and efficacy testing to protect farmers from fraudulent products in the market. Previously, N_2Africa and MIRCEN worked together in order to test commercial inoculants and offer quality assurance to their distributors and customers. In this respect, there is a need, particularly in Africa, to strengthen farmers’ capacities and establish networks for sharing reference protocols and information about BNF. Furthermore, very few firms in many African countries are involved in inoculum production and commercialization, limiting therefore access at adequate timely to quality inoculants.

4. Beneficial Mechanisms Other Than N-Fixation Provides by Diazotrophs Bacteria

In addition to their *N*-fixing abilities, diazotrophic bacteria are now recognized as also promoting plant growth (PGP) and yield and causing positive changes in soil structure and microbial community [38][39][40]. Many diazotrophic stains belonging to Rhizobia, Bradyrhizobia, Ensifer, Azotobacter, Azospirillum, Pseudomonas, Klebsiella, and Bacillus genera were reported to enhance the plant growth and grain yield of chickpea, bean, pea, wheat and rice through phytohormones and secondary metabolites production [40]. For instance, recent results from Gopalakrishnan et al. [41] have shown that rhizobia act also as PGP by producing indole acetic acid (IAA), siderophores, and organic acids, which leads to a stimulation of stems and roots growth of chickpea (*Cicer arietinum* L.). Some Bradyrhizobial strains isolated from rice rhizosphere and *Azorhizobium caulinodans* associated with *Sesbania rostrate* are capable of fixing nitrogen in the free-living state [42] under low-oxygen conditions [43]. Mia and Shamsuddin [44] have reported beneficial effects of rhizobium inoculation on different cereal crops as rice, maize, and wheat. On the other hand, Gopalakrishnan et al. [45] and Das et al. [46] reported that rhizobia can also act as biocontrol agents against pathogenic fungi (Rhizoctonia, Fusarium, Macrophomina, and Sclerotium), through hydrocyanic acid (HCN), antibiotics and/or mycolytic enzymes. The PGP traits of numerous other *α*-, *β*-, and *γ*-Proteobacteria inhabitants of legume nodules and contributing to N_2 fixation were always neglected. These new aspects of diastrophic bacteria, especially rhizobia are avenues for research in order to select efficient BNFs, for their better contribution in crop yield.

5. Synergistic Benefits

Soil microorganisms such as arbuscular mycorrhizal fungi (AMF) are known to have significant positive effect on BNF by direct and/or indirect interaction with *N*-fixing microorganisms. Indeed, AMF play a significant role in uptake of water and nutrients from soil [47] necessary to generate energy required for BNF [48] Moreover, through their hyphal networks, AMF can facilitate the colonization of legume roots by symbiotic *N*-fixing bacteria [49], as well as the transfer of nutrients and symbiotically fixed *N* between similar or dissimilar plants [50][51]. On the other hand, bacteria can also be beneficial to AMF. After characterizing a commercial AMF inoculum (AEGIS, i.e., Atens, Agrotecnologias Naturales S.L), Agnolucci et al. [52] showed that this product harbors many bacteria with important functional PGP properties such as nitrogen fixation, inorganic phosphate solubilization AIA production, etc. The synergic effects between AM fungi and soil microbial communities increase plant biomass and N acquisition from organic matter.

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