Case Study B2:

Overview and Case studies on Biological Nitrogen Fixation: Perspectives and Limitations

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Problem statement:

Biological Nitrogen Fixation and Sustainable Agriculture

Nitrogen is an essential plant nutrient. It is the nutrient that is most commonly deficient in soils, contributing to reduced agricultural yields throughout the world. Nitrogen can be supplied to crops by biological nitrogen fixation (BNF), a process which is becoming more important for not only reducing energy costs, but also in seeking more sustainable agricultural production. Nitrogen fixing micro-organisms could therefore be an important component of sustainable agricultural systems.

There are several significant reasons to seek alternatives to fertilisers that provide chemically fixed nitrogen:

- *Environmental*
  Nitrogen fertilisers affect the balance of the global nitrogen cycle, and may pollute groundwater, increase the risk of chemical spills, and increase atmospheric nitrous oxide (N_2O), a potent "greenhouse" gas.
- *Energy*
  The primary energy source for the manufacture of nitrogen fertiliser is natural gas together with petroleum and coal. On the contrary, the energy requirements of BNF are met by renewable sources such as plant-synthesised carbohydrates rather than from non-renewable fossil fuels.
- *Sustainability*
  Long- term sustainability of agricultural systems must rely on the use and effective management of internal resources. The process of BNF offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources.
- *Nutrition*
  It is estimated that about 20% of food protein worldwide is derived from legumes. There are more than 13,000 described species of legumes and for only 3,000 species examined more than 90% were found to form root nodules. Because few have been exploited for food, there is the prospect that the utilisation of legumes could be expanded substantially. It is anticipated that increasing demographic pressure and food demand will require the exploitation of BNF as a major source nitrogen for plant protein production.

Objective:

The objective of this paper was to explore and discuss the possibilities for enhancing N_2 fixation by working on the plant host, the microbial symbiont and management of different agronomic methods. Examples will be taken from research work across different agro-ecological and socio-economic contexts that illustrate best practices and experiences for enhancing biological nitrogen fixation.

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Strategies to enhance BNF in agricultural systems

There are several methods available to scientists working on enhancement of N_2 fixation:

1. *Host plant management (breeding legumes for enhanced nitrogen fixation)*
2. *Selection of effective strains able to fix more nitrogen*
3. *Use of different agronomic methods that improve soil conditions for plant and microbial symbionts.*
4. *Inoculation methods*

No one approach is better than the others, combining experience from various disciplines in inter-disciplinary research programmes should be pursued.

1. Host plant management

1.1. Plant selection

The amount of nitrogen fixed by legumes varies widely with host genotype, *Rhizobium* efficiency, soil and climatic conditions and, of course, the methodology used in assessing fixation.

The effectiveness of various legume species and their micro-symbionts has been provided in several publications[2]. The nitrogen fixing potential of a number of different legume species and their microsymbionts is showed in Figure 1.

Case 1 illustrates that when effective rhizobial populations are present either naturally or from inoculation, and there are no other major yield-limiting factor, plant selection is a potential method to enhance BNF.

Case 1: Genotypic variation in BNF by Common Bean
(adapted from Hardarson et al., 1993)[2]

The objective of this study was to investigate the N_2 fixation potential of various cultivars and breeding lines of common bean and to identify lines, which could be used as parents in breeding programmes to enhance N_2 fixation in this species.

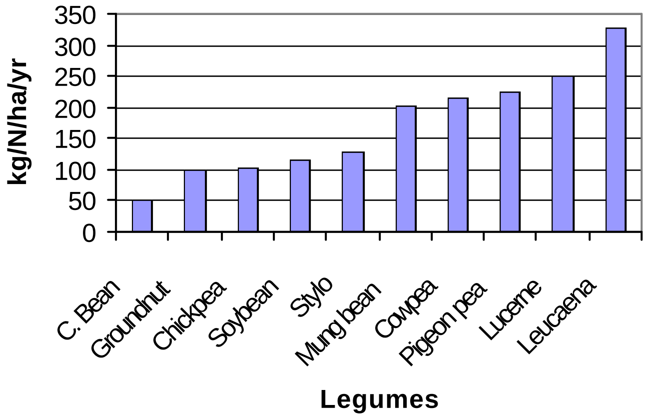
Field experiments were performed in Austria, Brazil, Chile, Colombia, Guatemala, Mexico and Peru as part of an FAO/IAEA Co-ordinated Research Programme to investigate the nitrogen fixing potential of cultivars and breeding lines of common bean (*Phaseolus vulgaris* L.). Each experiment included different bean genotypes, which were compared using ^{15}N-isotope dilution method (Table 1).
The results from the different countries showed that dry conditions and high temperatures contribute to low levels of fixation. Similar to other published results this study provides evidence for substantial genotype variability. The high values for nitrogen fixation were observed on adapted cultivars and breeding lines when the environmental conditions were favourable.
These can be used either directly as cultivars for production or in breeding programmes to enhance nitrogen fixation in their cultivars.
More effort in bean improvement programs should be placed on selection for increased nitrogen fixation under representative field conditions and involving improved inoculant when possible.

Table 1: Data from the FAO/IAEA Co-ordinated Research Programme to investigate the nitrogen fixing potential of cultivars and breeding lines of common bean (*Phaseolus vulgaris* L).

COUNTRY	Common bean tested (N°)	Total N fixed (Kg.ha^{-1})	Selected cultivars
Austria Seibersdorf)	29	25-165	Riz 44 ,Bat 322
Brazil (Goiania)	17	4-12	Honduras 35 Carioca
Chile	7	10-50	Red Mexican INIA Don Timoteo
Colombia (CIAT)	9	20-35	A268
Guatemala	10	92-125	ICTA San Martin ICTA Panamos ICTA Quenack-Ché
Mexico	18	0-70	Azufrado Negro Colima Negro Poblano
Peru Summer Winter	20	12-59 19-59	Summer: Cabalero, Caraota, Blanco. Winter: Bayo Normal, Canario G-62-2-6, Bayo G-7.5-9.

[2] *Hardarson, G; Danso, SKA; Zapata F; 1987. Biological nitrogen fixation in field crops. In: Handbook of Plant Science in Agriculture (Eds.) BR. Christie. CRC Press Inc. Boca Raton, FL, pp 165-192*
[2] *Hardarson, G., Bliss, F.A., Cigales-Rivera, M.R., Henson, R.A., Kipe-Nolt, J.A., Longeri, L., Manrique, A., Peña-Cabriales, J.J., Pereira, P., Sanabria, C.A., Tsai, S.M., 1993. Genotypic variation in biological nitrogen fixation by common bean. Plant Soil 152, 59-70.*

Figure 1. Average amounts of nitrogen fixed by various legumes (kg N/ha/yr)[3]



1.2. Plant improvements: roles for biotechnology

Although the direct molecular modification of a host plant or microsymbiont has yet to result in the improvement of N_2 fixation at the field level, several approaches offer promise. They include:

- *Host transformation to modify host range*. In a recent study transgenic *Lotus* plants transformed with the soybean lectin gene became susceptible to infection by *Bradyrhizobium japonicum* [4]

- *Host modification to synthesize opines*. Because *Rhizobium* strains vary in their ability to use opines, genetic engineering of legumes or other plants for opine synthesis may result in the enhancement growth of rhizosphere organism with the ability to utilise this substrate[5].

Case 2: Mutation breeding of grain legumes: an opportunity to enhance BNF
(adapted from Micke, 1993)[6]

The Objective of this work is to help plant breeders to develop improved cultivars through the use of induced mutations.
For example many mutants varieties of common bean have been released that possess many different improved characters (Table 2. Adapted from Micke , 1993). Mutation breeding therefore appears to be an appripriate approach not only for genetic improvement of grain legumes in general but also for improving their symbiotic nitrogen fixation.

Table 2. Improved cultivars of common bean developed by mutation breeding

Species and name of cultivars	Country and year of release	Mutagen used	Improved characters
Phaseolus vulgaris L. (common bean)			
Universal	Germany, 1950	x-rays	Early maturing, higher yield, disease resistant
Sanlac	USA, 1956	x-rays	Bush type
Urima	Germany, 1957	x-ray & cross	Disease resistant
Seaway	USA, 1960	x-rays & cross	Bush type, short duration, virus resistant
Gratiot	USA, 1962	x-rays & cross	Bush type, stiffer stem, higher protein content
Sapake 75	USSR, 1967	Gamma rays	Higher yield, better disease resistant
Seafarer	USA, 1967	x-rays & cross	Very early maturing, bush type, disease resistant
Pusa Parvati	India, 1970	x-rays	Early, bush type, higher yield
Alfa	CSFR, 1982	EMS	White seed colour, higher yield and protein content
Giza 80	Egypt, 1980	Gamma rays	Higher yield, larger grain, rust resistant
Muhranula	USSR, 1982	EI	Earlier
Ouray	USA, 1982	x-rays & cross	Bush type, better disease resistant
Makovskaya 8	USSR, 1985	Gamma rays	White seeds, earlier
Mogano	Italy, 1985	EMS	Uniform seed colour, dwarf type, high yield
Montalbano	Italy, 1985	EMS	Uniform yield colour, high yield
CAP 1070	Brazil, 1986	Gamma rays	Bush type, earlier maturity
Mitchell	Canada, 1986	x-rays & cross	Higher yield
Neptune	USA, 1986	x-rays & cross	Bush type

- *Genetic transformation of plants for enhancement malate dehydrogenase (MDH) synthesis in roots and nodules.* Malate is the primary plant carbon source used by bacteroid, and is also a factor in plant adaptation to P and Al stress. Alfalfa transformed

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[3] *FAO 1984. Legume Inoculation and Their Use. Food and Agriculture Organization of the United Nations. Rome 63 p.*
[4] *Van Rhijn, P., Goldberg, R.B., Hirsch, A.M., 1998. Lotus corniculatus nodulation specificity is changed by the presence of soybean lectin gene. Plant Cell 10, 1233-1249*
[5] *Savka, M.A., Farrand, S.K., 1997. Modification of rhizobacterial populations by engineering bacterium utilization of a novel plant produced resources. Nature (Biotech.) 15, 363-368*
[6] *Micke, A., 1993. Mutation breeding of grain legumes. Plant Soil 152, 81-85*

with a MDH gene having high efficiency in malate synthesis, exuded more organic material into the rhizosphere and fixed more N_2 than the wild type in initial studies (Temple *et al*., unpublished). Whether this also translates into enhanced P uptake and Al balance, remains to be determined.

- *Host mutants with improved characters such as disease and insect pest resistant, earlier and later flowering, higher yield, higher protein content or less toxic compounds (see Case 2)*[7].

2. *The effect of the micro-symbiont*

There are several important characteristics to be considered for the selection of the rhizobial-symbiont.

- *Nitrogen fixation ability:*

The rhizobia involved in nodulation can influence the percentage and amount of nitrogen fixed by the legume/*Rhizobium* symbiosis.
There are several methods available to quantify and estimate N_2 fixation. Plant dry weight is usually well correlated to effectiveness in N_2 fixation, when N is the only limiting growth factor. ^{15}N-based methods[8] provide direct evidence for N_2 fixation and can be used by developing countries largely through collaborative arrangements with developed countries and agencies that have the resources. The International Atomic Energy Agency (IAEA) Vienna, Austria assists developing countries through co-ordinated BNF programs. Results of a Rhizobium screening programme in India is illustrated in Case 3

- *Competitive ability:*

The proportion of the nodules formed on a particular host is influenced by the competitive ability of an inoculated *Rhizobium* strain in comparison to indigenous strains, which may vary in their effectiveness.

Case 3: Outputs on Rhizobium screening programme in India
(adapted from Khurana et al., 1998) [9]

Objective: to determine the effect of various factors such as the presence of a native homologous rhizobial population, soil mineral nitrogen, soil temperature and moisture, soil pH and interaction of rhizobia with other soil microbial communitieson the response of legumes to rhizobial inoculation.
Efficient strains of rhizobia perform extremely well under controlled conditions, however, the response to inoculation under field conditions is highly variable.
Selection of native effective Rhizobium strains was performed from diverse geographic regions in India. The response of rhizobial inoculation on chickpea grain yield was tested under the All India Co-ordinated Project on Improvement of Pulses (AICPIP) under the aegis of the Indian Council of Agricultural Research (ICAR).
Data on the response of rhizobial inoculation on chickpea yield during three years 1993-95, 96 at 38 farmer's fields in seven states is summarised in Table 1 (Khurana *et al*., 1999).
In traditional chickpea-growing areas in India it was observed that about 18% farmer's fields had <102 rhizobia g^{-1} soil. Significantly improved yield due to rhizobia inoculation is expected when a field has < 102 rhizobia g^{-1} soil and other factors affecting BNF are optimum

Table 1. Residual maximum likelihood estimates of grain yield in on-farm experiments on rhizobial inoculation in chickpea, 1993-1996, at various locations in India.

Location	Year	N° farmers fields	Grain yield ($kg\ ha^{-1}$)		Increase over control ($kg\ ha^{-1}$)
			Non-inoculated	Inoculated	
Maharashtra	1995-96	13	938	1026	88
Rajasthan	1993-94	5	1068	1212	144
	1994-95	3	1731	2120	339
	1995-96	6	897	1015	118
Karnataka	1994-94	1	840	1350	510
	1994-95	2	755	900	145
Haryana	1994-95	2	1100	1300	200
	1995-96	3	1367	1533	168
Punjab	1993-94	5	642	752	110
	1994-95	5	1750	2020	270
Uttar Pradesh	1993-94	1	847	1007	160

Other important characteristics of rhizobial inoculants are:

i. Survival ability
ii. Colonisation of the rhizosphere
iii. Migration in the soil

[7] *FAO/IAEA 1988. Improvement of Grain Legume Production Using Induced Mutations. IAEA, Vienna.*
[8] *Hardarson, G., Zapata, F., Danso, S.K.A., 1984. Field evaluation of symbiotic nitrogen fixation by rhizobial strains usin ^{15}N methodology. Plant Soil 82, 369-375*
[9] *Khurana, A. L., Dudeja, S.S., Sheoran, A., 1998. Biological nitrogen fixation in chickpea for sustainable agriculture. Prospects and limitations. Sust. Agric. Food, Energy, Ind.439-444*

Rhizobial inoculant for a particular legume species can be obtained either from other research laboratories, or through a selection programme. Selection of Rhizobia is justified only when no suitable strain is available from other sources.
Strain selection will be required for example:

- *when the legume of interest is an uncommon species for which there is no recommended strain and,*
- *when inoculation with a recommended strain does not produce adequate nodulation and fixation.*

2. *Factors, affecting BNF: Management decisions*

Environmental factors affecting nitrogen fixation include temperature, moisture, acidity and several chemical components of the soil such as nitrogen, phosphorus, calcium and molybdenum content [3]. It is often difficult to isolate the effect of the above factors on inoculation success from their influence on symbiosis and nitrogen fixation. For example: acidity, as well as, calcium, aluminium and manganese concentrations will interact and affect both bacterial proliferation, root-hair infection and plant growth [3].
Numerous (micro)-climatic variables, soil physical properties and agronomic management factors also play a part in controlling N_2 fixation; however, none of those factors should be considered in isolation as all are interconnected in the control of N_2 fixation (Figure 2).

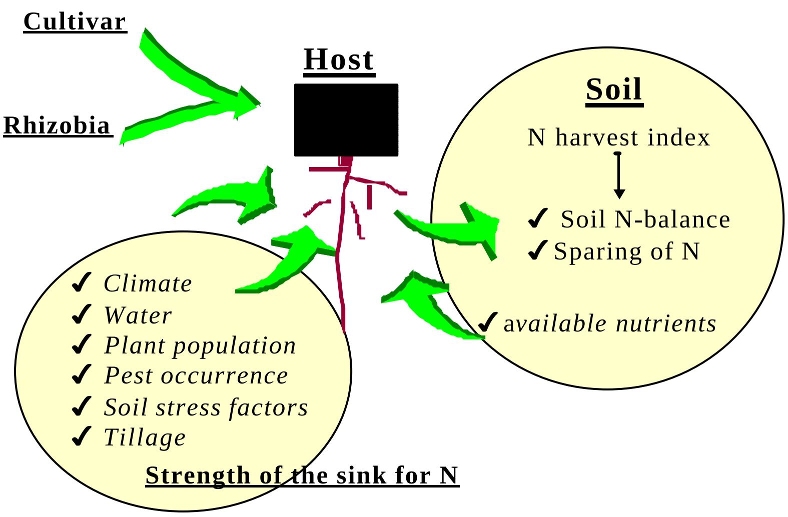

Figure 2. Conceptual model of the major factors that exercise a control on N_2 fixation of grain legumes in a cropping system (adapted from van Kessel & Harley, 2000)[10]

In addition to the competitiveness of the rhizobia in forming nodules and the effectiveness of the rhizobium-host plant to fix N_2, a series of edaphic, chemical and biophysical factors exert a control on N_2 fixation. Management practices like the intensity of tillage or intercropping practices will alter those edaphic, chemical and biophysical factors and therefore influence BNF indirectly as illustrated in Table 4.

[10] *Van Kessel, C., Hartley, C., 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation?. Field Crop* Res. 65, 165-181

Table 4. Factors Affecting Biological Nitrogen Fixation: a summary of some important factors limiting biological nitrogen fixation and possible recommendations .

Factors affecting BNF	Effect	Recommendations
Temperature & moisture	• survival, of rhizobia in soil [11] • abilities to nodulate and fix nitrogen [12 13] • inhibition of nitrogen fixation[14]	• placement of inoculum in deeper soil layers when top soil temperature are high [15] • the surface mulches may conserve moisture and reduce soil temperature [15]
Nitrogen Fertilisation	• Generally combined N delays or inhibits nodulation and nitrogen fixation. Because of this adverse effect, N fertilisation usually is not recommended for leguminous crops. However there may be situations where N has to be applied, such as to cereals in mixed cropping or rotations and then fertiliser may affect nitrogen fixation of the legume crop. [16]	• the development of grain legumes which are less sensitive to mineral N should not be pursued unless there is increase in N-uptake and an improvement in the overall use efficiency of available N. [16] • it is possible to apply small amounts of soil or foliar N fertiliser, which may increase yield without reducing the amount of nitrogen fixed.[17, 18]
Pesticides, fungicides and insecticides	• The compatibility of rhizobia with pesticides is poorly understood except for fungicides.[3],[19] • Insecticides have little adverse effect on nodulation when not directly applied on seed. • The effect of herbicides on rhizobial survival is unknown.	• due to the variability of the effect it is recommended to test the particular Rhizobium inoculum and its behaviour in respect to the product to be used, before application • the effect of pesticide on N fixation should be minimised by separate placement of rhizobia and pesticide
Intercropping	• Increase opportunities for N-use complimentarily, reducing the need for fertiliser-N, either by increasing the availability of soil N or by N transfer.	• Several examples producing mixed results are showed in Case 4 on Intercropping management
Acid soils	• Acid soils constrain agricultural production and nitrogen fixation.	• Use of acid-tolerant legume cultivars and rhizobium • Soil liming should be limited to achieving a pH at which available aluminium or manganese levels are no longer toxic.
Tillage	• When tillage is minimised, lower rates of mineralization and nitrification, coupled with increased N immobilisation and a higher potential for denitrification will lead to a decrease in available N.	• Limiting tillage can stimulate N demand and N_2 fixation. Conservation and zero tillage management practices will, therefore, lead to a stimulation of N_2 fixation, at least until a new equilibrium between residue input and the rate of decomposition is reached. Results from several field experiments are showed in Case 5 on Tillage management.

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[11] *Bowen, G.D., Kennedy, M.M., 1959. Effect of high soil temperature on Rhizobium spp. Qld. J. Agric. Sci. 16, 177-197*
[12] *Hardarson, G., Jones, D.G., 1979. Effect of temperature on competition amongst strains of Rhizobium trifolii for nodulation of two white clover varieties. Am. Appl. Biol. 92, 229-236*
[13] *Montañez, A., Danso, S.K.A., Hardarson, G., 1995. The effect of temperature on nodulation and nitrogen fixation by five Bradyrhizobium japonicum strains. App. Soil Ecology2, 165-174*
[14] *Hungria, M., Franco, A.A., 1993. Effect of high temperature on nodulation and nitrogen fixation by Phaseolus vulgaris L. Plant Soil 149, 95-102.*
[15] *Roughley, R.J., 1980. Environmental and cultural aspects of the management of legumes and Rhizobium. In: Advances in Legume Sciences (Eds.) R.J. Summerfield and A.H. Bunting pp. 97-103. Royal Botanic Gardens, Kew*
[16] *Hardarson, G., Danso, S.K.A., Zapata, F., Reichardt, K., 1991. Measurements of nitrogen fixation in favabean at different N fertiliser rates using the ^{15}N isotope dilution and A-value methods. Plant Soil 131, 161-168*
[17] *Boote, K.J., Gallagher, R.N., Robertson, W.K., Hinson, K., Hammond, L.C., 1978. Effect of foliar fertilization on photosynthesis, leaf nutrition and yield of soybean. Agron. J. 70, 787-791*
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[19] *Ramos, M.L.G., Ribeiro, W.O., Kipe-Nolt, J.A., 1993. Effect of fungicides on survival of Rhizobium on seeds and the nodulation of bean (Phaseolus vulgaris L.). Plant Soil 152, 145-150*

Case 4: Intercropping management
(adapted from van Kessel & Hartley, 2000)[10]

The total amount of N fixed per unit area in intercropped systems is often lower due to decreased legume population densities, and increased competition for light and nutrients by the non-legume. An increase in the total amount of N_2 fixed could occur when the intercropped legume uses more effectively limited resources

Table 5. Variation in the N_2 fixation by grain legumes grown in monoculture (M) or intercropped (I) with non-legumes. (Cropping densities and fertilisation rates were not specified).

Cropping system	N_2 fixed (%)		N_2 fixed (kg ha^{-1})	
	M	I	M	I
Soybean/non-nodulating soybean [20]	42	23	71	17
Pea/barley [21]	62	84	115	81
Cowpea/maize [22]	28	34	22	10
Pea/mustard [23]	48	50	71	62
Pigeonpea/sorghum [24]	74	55	169	124
Pea/oats [25]	27	52	22	30
Lentil/flax [26]	77	85	14	8
Pea/rape [26]	38	33	41	27
Pea/mustard [26]	28	34	20	18
Pea/oats [26]	80	86	50	16
Pea/rape [26]	78	88	20	27
Ricebean/maize [27]	32	75	30	39
Cowpea/rice [28]	32	30	35	32
Fababean/barley [29]	74	92	79	71
Pea/barley [30]	68	84	213	74

Case 5: Tillage management
(adapted from van Kessel & Hartley, 2000)[10]

Results from various field experiments with grain legumes are shown in Table 6 (adapted from van Kessel & Hartley, 2000).

Table 6. Influence of conventional (CT) and zero tillage/minimum tillage (ZT/MT) practices on N_2 fixation by grain legumes.

Crop	N_2 fixed (%)		N_2 fixed (kg ha^{-1})	
	CT	ZT/MT	CT	ZT/MT
Chickpea [32]	34	28	32	27
Soybean [33]	73	88	180	232
Soybean [34]	73	88	91	156
Chickpea (1994) [35]	31	40	9	11
Chickpea (1995) [35]	12	17	4	5
Pea [36]	48	79	ND	ND
Lentil [36]	62	72	ND	ND
Soybean (Cultivar S12) [37]	87	91	33	47
Soybean (Cultivar S15) [37]	86	88	39	44

** ND: not determined.*

3. Inoculation

Inoculum strains when applied to the target ecosystem have to compete with all of the negative and neutral microbes presented in the soil. This competition could reduce the efficacy of the final product and therefore methods and strategies to improve *Rhizobium* performance should be studied.

3.1. Determining the need for inoculation[31]

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[29] Danso, S.K.A., Zapata, F., Hardarson, G., Fried M., 1987. Nitrogen fixation in favabeans as affected by plant population density in sole or intercropped systems with barley. Soil Biol. Biochem. 19, 411-415

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In many soils, the nodule bacteria (*Rhizobium spp*) are not adequate in either number or quality. Under these conditions, it is necessary to inoculate the seed or soil with highly effective *Rhizobium* cultures.

1- Inoculation is almost always needed when certain new leguminous crops are introduced to new areas or regions. Host-specific rhizobia are frequently developed for new cultivars or varieties of legumes

2- Many soils are heavily infested with ineffective rhizobia capable of inducing nodulation without host benefit. Under such conditions, a very large inoculum of competitive and highly effective strain of rhizobia is needed to replace the ineffective native rhizobia.

Legume response to inoculation [37 38] *was largely dependent on:*

- *number of rhizobia already established in the soil*
- *availability of soil N*
- *demand for N by the crop*

Case 6: The benefits of inoculation
(adapted from Singelthon et al., 1992) [31]

Objective was a comprehensive, five-year effort by NifTAL (Nitrogen Fixation in Tropical Agricultural Legumes) to determine the benefits of inoculation for agriculturally important legumes. The results showed clear benefits from inoculation.
In 228 standardised field experiments covering more than 20 countries and 19 species of legumes, the majority of the trials showed a significant response (<1.0 S.D) to inoculation, both when the trials were conducted in farmers' fields and under more intensive management and higher inputs (Table 5)

Table 5. Rhizobial inoculation and the yield response of tropical legumes

Species	Total N° of trials	Significant response to inoculation (% of total)	
		Low inputs managem ent	High inputs management
Peanut	26	50	46
Chickpea	31	48	55
Pigeonpea	8	13	13
Soybean	40	65	65
Lentil	27	48	41
Bean	10	10	30
Gram (black)	15	53	60
Mung bean	40	70	68
Cowpea	9	56	11

Clearly, there is a yield advantage to inoculation. However, the yield responses to inoculation were highly variable and affected by inherent field variability, even in the small-plot field experiments, and by differences in environmental and edaphic conditions

3.2. Enhancing the effectiveness of inoculants

- <u>*Inoculation technology*</u>

The technology should aim at protecting the viability of the microorganism and helping them to occupy the target niches and to express their biological functions.

Examples

- Microcapsulation techniques have been successfully used to entrap biofertilizers agents in biodegradable polymers to protect them against storage conditions, oxidation, dryness, UV light and other environmental stresses[23]
- Sterile carriers (Gamma radiated or short wave) with lower water potential proved to help preconditioned biofertiliser inoculant strains to environmental stresses, as well as to support a higher microbial count with a long expiration date [24]

- *Inoculation methods*

Methods of rhizobial inoculation can have great influence on the amount of N_2 fixed. There are several considerations to be taken into account when optimising inoculation methods and these have been reviewed for FAO, 1984.

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[37] *Thies, J.E., Singleton, P.W., Bohlool, B.B., 1991a. Influence of size of indigenous rhizobial populations on establishement and symbiotic perrformance of introduced rhizobia on field-grown legumes. Appl. Environ. Microbiol. 57, 19-28..*
[38] *Thies, J.E., Singleton, P.W., Bohlool, B.B., 1991b. Modelling symbiotic performance of introduced rhizobia in field-based indices of indigenous populations size and nitrogen status of the soil. Appl. Environ. Microbiol. 57, 29-37*
[39]*Trevors, J., 1991. Appl. Microbiol. Biotechnol. 35, 416-419.*
[40]*Somasegaran, P., 1985. Appl. Environ. Microbiol. 50(2): 398-405.*

Example?

- It has been demonstrated that nodules on the lower part of the root system can fix more nitrogen over the whole growing season than the crown nodules, and they may contribute most of the nitrogen fixed by the legume plant[41]. Farmers applying inoculum on the seed can therefore not expect these bacteria to form nodules on the whole root system. It is likely that applied rhizobia form some or most of the nodules on the crown but other indigenous rhizobia in the soil may form the nodules at greater depth and distance from the crown. It should be possible to enhance N_2 fixation by promoting optimal production of nodules on lateral roots by selecting rhizobia not only for the effectiveness to fix N_2 but also for migration in the soil and along the root under a range of conditions.

➢ *Associative biofertilizer inoculants*

Associative nitrogen fixation inoculant has also been developed and commercially produced for wheat, barley, cotton, canola, sugarcane, maize, and vegetables. The output of these inoculants had been inconsistent and more site and crop dependent. Thus, co-inoculants will require extensive in-vitro and in-situ investigations if the positive attributes associated with each organism are to be effectively exploited.

Case 7: Associative biofertilizer inoculants

Table 6. Output of some associative biofertiliser inoculants - some succesful examples.

CROP	INOCULANT	Out put of application	Country
Soybean [42]	Rhizobium+ phosphorus-solubilizing bacteria+P fertiliser	Increase seed protein	India
Acacia nilotica [43]	Rhizobium & Mycorrhiza	Enhanced ancillary characters	India
Rice [44]	Azolla, green manure	10% increase in straw yield by 3x incorporation of Azolla	Egypt
Wheat [45]	A. lipoferum & B. polymxa	6% increase in grain yield	Argentina
Wheat [46]	Composite of nitrogen fixers Gram negative	17% increase in grain yield in new sandy land	Egypt
Sorghum [47] (forage)	Composite of nitrogen fixers Gram negative	I ncrease in dry matter 3-21%	Egypt
Wheat [48]	Azorhizobium	8.85% increase in nitrogen content using N15	China
Oak Forest [49]	Clostridium butyricum	8.2 kg N/ha/year	England
Wheat [50]	A. brasilense	Enhancing the accumulation of trace elements	USA

➢ *Role of biotechnology in enhancing the efficacy of inoculants*

Biotechnology and gene manipulation techniques were able to provide potential means to improve the commercial inoculant strains. During the last 10 years, extensive studies revealed the genetic determinants and the regulation pathways of most of the microbial functions. Genes that control nodulation (*nod, ndv*), nitrogen fixation (*nif, fix*), host range (*nod, hsp*), surface polysaccharide (*exo*) and energy

[41] *Hardarson, G., Golbs, M., Danso, S.K.A., 1989. Nitrogen fixation in soybean (Glycine max L. Merrill) as affected by nodulation patterns. Soil Biol. Biochem. 21, 783-787*
[42] *Sharma, K.N., Namdeo, K.N., 1999. Effect of biofertilisers and phosphorus on growth and yield of soybean (Glycine max.L).Crop Research 17, 160-163*
[43] *Harvir-Kaur, Pandher, M.S., Gupta, R.P., Garcha, H.S., Kaur, H., Dhaliwal, G.S. (Eds.), Arora, R. (Eds.) Randhawa, N.S. (Eds.), Dhawan, A.K. Effect of Rhizobium and VAM on Acacia nilotica in two soils of different agroclimatic regions. Ecological Agriculture and sustainable development, volume 1. Proceedings of international conference on ecological agriculture. India, 1997*
[44] *Yannie, Y.G., Shalaan, S.N., El-Haddad, 1993. 6th Int. Symp. On Nitrogen Fixation with Non-Legumes, Ismailia, Egypt, Sept. 1993*
[45] *Caceres, E.A.R., Anta, G.C., Ciocco, C.D., Basurco, J.C.P., Parada, J.L., 1993. . 6th Int. Symp. On Nitrogen Fixation with Non-Legumes, Ismailia, Egypt, Sept. 1993*
[46] *Abbas, T.M., Rammah, A., Monib, M., Ghanem, E.H., Eid, M.A., Emara, F.Z., Hegazi, 1993. 6th Int. Symp. On Nitrogen Fixation with Non-Legumes, Ismailia, Egypt, Sept. 1993.*
[47] *Helmy, A., Youssif, H., Rammah, A., Hanna, A., Eid, M.A., Bedawi, E.H.N., Hegazi, 1993. . 6th Int. Symp. On Nitrogen Fixation with Non-Legumes, Ismailia, Egypt, Sept. 1993.*
[48] *Xie, Y.X., Chen, W.H., 1993. 6th Int. Symp. On Nitrogen Fixation with Non-Legumes, Ismailia, Egypt, Sept. 1993*
[49] *Jones, K., Bangs, D., 1985. Soil Biol. Biochem. 17(5): 705-709*
[50] *Bashan, Y., Harrison, S.K., Whitmoyer, R.E., 1990. Appl. Environ. Microbio. 56(3): 769-775*

utilisation (*dct, hup*) have been identified. Inoculant strains were able to take advantage of these techniques to produce value-added inoculants.

4. CONCLUSIONS

Biological N_2 fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity. However, if BNF is to be utilised, it must be optimised. In the near future, particularly in developing countries, tremendous opportunities exist for enhancing the BNF capacity of legumes.

There is no simple and easy approach to increase BNF in grain legumes grown as part of a cropping system, under realistic farm field conditions.. Numerous (micro)-climatic variables, soil-physical properties, agronomic management, host-rhizobia combination and socio-economic aspects play an important role in controlling BNF.

- The use of improved host-rhizobia combination has great potential to increase N_2 fixation. Interaction between a range of traits and N_2 fixing symbiosis will require particular care in breeding and selection programs aimed at alleviating environmental and management practices that reduce BNF.
    - Programmes for host plant selection
    - Programmes for *Rhizobium* selection

- Management practices that increase N demand by the host plant is a promising avenue to increase N_2 fixation in grain legumes in a cropping system. The most likely practices to have an impact on BNF are:
    - Improving pest management practices
    - Improving soil structure
    - Conversion from conventional tillage to zero or minimal tillage
    - Improving the overall fertility status of the soil, while maintaining low levels of available soil N.

There are several methods available to enhance BNF, as shown in the present paper. No one approach is better than all others, rather work on symbiosis combining experience from various disciplines in interdisciplinary research programmes should be pursued.

USFUL CONTACTS

- ***On going research and projects related to N_2 fixation***

International research institutes

- Cowpea_Cereals Systems Improvement in the Dry Savannas
http://www.cgiar.org/iita/research/parpt/project11.pdf

- Improvement of Maize Grain Legume Production
http://www.cgiar.org/iita/research/parpt/project12.pdf

- FAO/IAEA Agriculture and Biotechnology Laboratory
http://www.iaea.org/programmes/nafa/d1/

Universities

- University of Reading. Faculty of Agriculture and Food.. Department of Soil Science
http://www.rdg.ac.uk/AcaDepts/as/home.html

- ***Collection of nitrogen-fixing bacterial legume symbionts***

- Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA)
http://bldg6.arsusda.gov/pberkum/Public/sarl/welcome.html