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Cover photograph (front) Field trial with plant growth promoting rhizobacteria of groundnut

Cover photograph (back): ICAR-DGR Office

Foreword

India is the largest producer of oilseeds in the world but still it depends on the import of edible oil. Groundnut contributes around 30% of the total edible oil requirement of the country. But it is not sufficient enough to meet the demand of the ever growing population. As the total acreage is not likely to increase further, productivity needs to be improved with an average annual growth rate of around 3%. The task is daunting owing to the fact that groundnut crop is grown in the country predominantly in rainfed situation in marginal lands, very often with suboptimal dose of external input of fertilizers. Even with the adoption of advanced technologies and high yielding varieties, the productivity of groundnut is fluctuating around 1270 kg/ha.

To improve the soil health, and for better transformation and mobilization of nutrients in soil, application of highly competitive strains of groundnut rhizobia, plant growth promoting rhizobacteria and other beneficial microbes is very crucial to enhance groundnut productivity. However, very often, biofertilizers which are available at affordable costs are not applied or products of good quality are not available. With the growing awareness of organic agriculture, the demand for biofertilizer is increasing but needs further popularization about the potential benefits the biofertilizers can provide to the farmers. This bulletin is an effort to fulfill this requirement. Over the years, the microbiologists of this directorate have developed a number of biofertilizers which have great potential in improving the productivity of groundnut in future. I hope the information provided in this bulletin will benefit the researchers, extension workers and farmers for popularizing and adopting the biofertilizer technology for enhancing groundnut productivity.

I wish to compliment the authors in bringing out this useful publication.

Radhakrishnan T.
Director, ICAR-DGR

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Preface

Groundnut is one of the major oilseed crops of the tropics and sub-opics, grown in an estimated area of about 25 million ha in about 90 ountries with an average yield of about 1600 kg. In India, groundnut is ultivated in around 6 million ha with an annual production of around 7 illion tonnes and with an average productivity of around 1270 kg/ha in ainfed situation. There is a fluctuating trend in area, production and roductivity of groundnut in India. As around 90% of the crop is grown nder rainfed conditions, productivity is affected due to erratic rainfall and equent occurrence of droughts.

One of the reasons of poor productivity in India is the cultivation of roundnut in marginal lands with suboptimal nutrient supply. However, eing a legume crop most of its N requirement should be met from iologically fixed nitrogen. But promiscuity of groundnut allows nodulation y all the different strains of rhizobia present in the tropics and sub-tropics esulting in erratic biological nitrogen fixation. The situation is ompounded further as native rhizobia often out-compete the inoculants trains. Because of insufficient biological nitrogen fixation in groundnut, ery often yield is badly affected. Thus, application of highly competitive nd efficiently nitrogen fixing strains of groundnut rhizobia would help in nhancing the biological nitrogen fixation vis-à-vis groundnut yield.

The soils of groundnut growing areas are very often affected by cidity or alkalinity coupled with deficiency in available P, Fe, Zn, Mo, and ther micronutrients. Therefore, with the application of phosphate olubilizing microorganisms, plant growth promoting rhizobacteria and ther beneficial bacteria having multiple plant growth promoting traits, ansformation and availability of P, Fe, Zn, Mo and other micronutrients an be improved substantially. Biofertilizers hold promise in improving he productivity of groundnut in a sustainable way, though their potential in creasing yield has not been exploited yet.

With the sincere efforts of the microbiologists of the ICAR-irectorate of Groundnut Research, a number of highly competitive trains of groundnut rhizobia, plant growth promoting rhizobacteria, onsortia of beneficial bacteria and phosphate solubilizing bacteria have een identified and their potential demonstrated throughout the country hrough FLDs and demonstration. There is a need for further opularization of the biofertilizers. To provide valuable information to the armers and extension worker about the biofertilizers of groundnut and the enefits that can be accrued from biofertilizers usage, this bulletin has een compiled. Hope this will benefit the groundnut growers of the ountry.

Rinku Dey
K. K. Pal

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1. Introduction

Groundnut is cultivated in around 6 million ha with an annual production of around 7 million tonnes in India. However, the productivity remains a matter of great concern as it remains around 1250 kg/ha in rain-fed situations and around 1800 kg/ha in irrigated conditions. As 90% of the crop is grown under rainfed conditions, productivity is affected by erratic and uneven distribution of rainfall, and in odd years the crop is badly affected by drought in most part of the country.

In India, groundnut is mostly cultivated in marginal lands with suboptimal nutrient supply. Being a legume crop most of its N requirement should be met from biological nitrogen fixation. With an average pod yield of 2 t/ha, there is requirement of about 250 kg nitrogen. However, in rainfed and irrigated conditions, it is recommended to apply only 12.5 kg and 25 kg N/ha, respectively, and thus there is a requirement of about 200 kg of N being fixed by biological nitrogen fixation. However, groundnut is a promiscuous crop and is often nodulated by all the strains of rhizobia present in the tropics and sub-tropics. The situation is compounded further as native rhizobia often out-compete the inoculants strains. Thus, there is highly erratic biological nitrogen fixation in groundnut. Because of this, there is acute imbalance in the nitrogen availability in groundnut, thus affecting the productivity.

Marginal lands are mostly deficient in available phosphorus and an external application of P_2O_5 @ 40 kg /ha is recommended for groundnut cultivation. However, in acid soil, P is fixed as Fe- and Al-phosphates and in alkaline soil it is fixed as tri-calcium phosphate. Thus, the P requirement of groundnut is seldom realized. To improve the phosphorus nutrition, inoculation with phosphate solubilizing microorganisms is helpful. The soil is also often deficient in micronutrients in different parts of the country, specially Fe, Mo, Zn, etc.

Thus, to enhance the productivity of groundnut, application of highly competitive strains of groundnut rhizobia, phosphate solubilizing microorganisms, plant growth promoting rhizobacteria (PGPR), AM fungi and other beneficial bacteria like endophytes would help in realizing good yield in a sustainable manner.

2. Biofertilizers in agriculture

Intensive commercial farming involves excessive use of agricultural land area through multiple cropping and uses of chemical fertilizers and pesticides as well. In the recent past good quality of chemical fertilizers and pesticides have been employed in farmlands. It is feared that practice of uses of chemical fertilizers and pesticides continually would result in gradual decrease in quality of soils especially in terms of fertility. Use of agriculturally important microorganisms in different combinations is the only solution for restoration of soils.

In order to increase the unit area productivity of agricultural land, the role of different crop nutrients in contributing increased crop yield play a vital role. Among the crop nutrients, nitrogen as well as phosphorus plays an important role in increasing the crop productivity. Further, the nitrogenous chemical fertilizers are manufactured industrially using non-renewable petroleum products under high temperature and high pressure. Increase in petroleum cost day by day affects the cost of the chemical fertilizers. On the other hand when increased doses of chemical fertilizers are used to increase crop production it causes environmental pollution and is also toxic to soil as it kills the beneficial microorganisms. Plant nutrients like N, P and K are highly essential for plant growth. The plants remove many nutrients from the soil in modern intensive cultivation and needs replenishment. Under such conditions microbes offer good alternative technology to replenish crop nutrients.

In agricultural eco-system, microorganisms are having vital role in fixing/solubilizing /mobilizing /recycling nutrients. These microorganisms occur in soils naturally, but their populations are often scanty. The beneficial microbial population occurring in soils always may not be supporting for higher crop yield. In order to increase the crop yield, the desired microbes from rhizosphere are isolated and artificially cultured in adequate count and mixed with suitable carriers. These are known as biofertilizers or microbial inoculants. It includes *Rhizobium, Azotobacter*, *Acetobacter*, *Azospirillum*, phosphate solubilizing microorganisms (PSM), arbuscular mycorrhizae (AM fungi), plant growth promoting rhizobacteria (PGPR), micronutrient mobilizing bacteria like *Thiobacillus* sp., etc.

These biofertilizers are microbial inoculants, which contain living cells of efficient nitrogen fixing microorganisms, which fix atmospheric nitrogen either symbiotically with host plant or free living, phosphate solubilizing /mobilizing microorganisms as well as potassium mobilizers /release or mobilize P_2O_5 and K_2O from soil. These biofertilizers are available in markets as carrier based inoculants or as liquid inoculants.

Biofertilizers are inexpensive and eco-friendly. Many State Agricultural Universities, Govt. Agriculture/Forest Departments and a good number of commercial units in private and public sectors are producing and distributing biofertilizers.

Formulation comprises aids to preserving organisms and to delivering them to their target fields and – once there –to improving their activities. A technical concentrate of an organism that has been achieved by a particular process is called as a formulation, or a product, which may be stored and put on sale commercially.

3. Why biofertilizers?

- To enhance the efficiency of the externally applied nutrients
- For transformation, mobilization and availability of the fixed or unavailable forms of nutrients
- To sustain crop production, maintain soil health and soil biodiversity in the long run
- To integrate biofertilizers, biocontrol agents and organic materials in farming system to provide stability and sustainability in production
- To promote eco-friendly agriculture and organic farming

4. Chemical fertilizers vs biofertilizers

Excessive and indiscriminate use of chemical fertilizer may lead to:

- Decrease in organic carbon in soil
- Decrease in population and diversity of beneficial soil microflora
- Increase in soil acidity, alkalinity and hardening
- Excessive use of N fertilizers may affect ground water and human and animal health
- Environmental pollution

- Deterioration in soil health
- Imbalance in soil nutrient status

Though effect of microorganisms is slow, it is sustainable in long term basis and would provide following benefits:

- May increase crop yield upto 15%
- Reduce external application of N and P by 25%
- Stimulate plant growth
- Slow but long term effect
- Activate soil system biologically
- Restore natural fertility
- Provide protection against drought and some soil borne fungal pathogens
- Low cost
- Easy to use
- Eco-friendly
- Maintain soil biodiversity

5. Types of formulations

There are varieties of formulation both in liquid and solid forms. The main types currently used for biofertilizers have been classified into dry products (dusts, granules and briquettes) and suspensions (oil or water-based and emulsions). A wide range of formulations with additives is available in market.

(a) Dry inoculum products

These formulations comprise dusts, granules and briquettes, based on particle or aggregate size. Wettable powders are also included in this group, which are formulated as dry powder mixed in water as a carrier, just before use. Dusts mainly contain 30% of an organism by weight.

(b) Granules, pellets, capsules and briquettes inoculum

Granules are discrete masses 5-10 mm^3 in size, pellets are >10 mm^3, and briquettes are large blocks up to several cubic centimeters; like dusts, these products contain an inert carrier holding the organisms. Carriers include clay minerals, starch polymers and ground plant residues. Soft carriers, e.g. Bentonite, disburse quickly to release the organism. The product can be coated with various materials to slow down or control the rate of release, which also depends on unit size. The concentration of organisms in granules is 20-30%.

(c) Wettable powders inoculum

This formulation is predominant among all commercial products and comprises charcoal, lignite, vermiculite powders blended with 3% gum to make them stable during storage on the shelf and readily stick with seeds.

(d) Liquid formulations

Liquid biofertilizers are special liquid formulation containing not only the desired microorganisms and their nutrients, but also special cell protectants or substances that encourage formation of resting spores or cysts for longer shelf life and tolerance to adverse conditions.

Temperature is important for the shelf life of microbial products and it can affect their activity before or after application. Colonization proceeds at field temperatures in the cropping season, but slows at lower or higher temperatures.

Bacteria used for plant growth need the plant surface to be wet in order to establish them. These needs can be overcome by liquid formulation as products contain humectant.

It is always mentioned on the packet of microbial inoculum that it must be kept away from direct sunlight. To counter harmful effects of sunlight, sunscreens are added to liquid formulations.

The pH of a product plays a vital role in liquid inoculum preparations. A buffer therefore is maintained by adding some additives, which render better shelf life in liquid.

6. Carrier-based inoculants

In India, peat-like material available in the Nilgiri valley has been found to be a good carrier. Lignite and charcoal are also used widely as carrier materials.

Culture broth is grown in a suitable medium in large flasks on a shaker for small requirements or in fermentors for large requirements. The powdered carrier (passing through 100 mesh sieve) is neutralized with $CaCO_3$ and autoclaved at 15 lbs pressure for 4 hours. After cooling, a high-count broth is mixed so as to attain a 40% moisture-holding capacity of the carrier. The carrier and the broth are mixed either manually or by means of a mechanical mixer, cured in trays for 2-5 days, and packed in polythene bags.

7. Method of application

(a) Seed treatment

The seeds can be coated with either carrier based or liquid based inoculums in following manner in groundnut (Figure 1). The size of the container will depend on the amount of the seed required for sowing as well as seed size. Generally, biofertilizer of groundnut can be mixed with the seeds in a plastic container as the size of seed is large in groundnut as compared to other agricultural commodities. The use of 10 % sugar or 40% gum Arabic in the suspending fluid enhances the survival of rhizobia on seed.

Carrier- based cultures are mixed with minimum amount of water to form a slurry (sugar or gum Arabic, may be added) and seeds are added to the slurry so as to uniformly coat the seeds with the inoculants. The seeds are dried in shade and sown immediately.

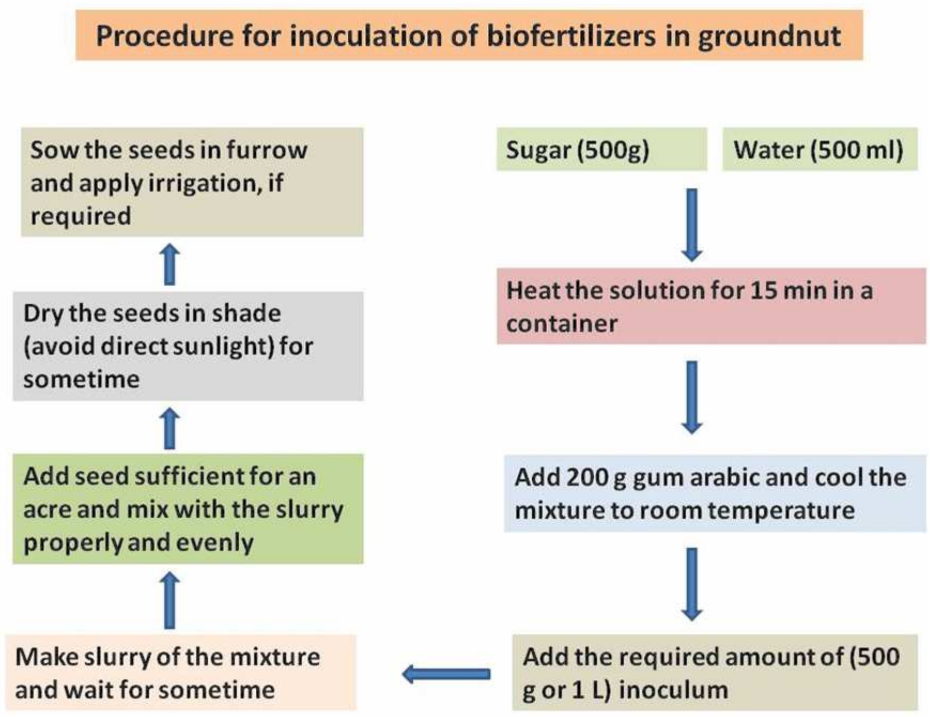
(b) Through FYM

For even distribution and spread over large area, biofertilizers can be applied after mixing with the FYM but sufficient moisture must be maintained to prevent drying up of the inoculums otherwise the inoculums load will go down. In groundnut, FYM enriched with biofertilizers is applied in set furrows and then seeds are sown in the furrows and irrigation is provided.

(c) Through irrigation water

Liquid biofertilizer diluted sufficiently can also be applied in irrigation channels to spread all over the field uniformly. This will also maintain viability of the cells and prevent desiccation death of the live biofertilizer cultures.

Figure 1. Method of coating of groundnut seeds with biofertilizers



8. Quality control

In India, the Indian Standards Institution ISI has evolved methods to check the quality of inoculants and issue ISI marks to qualified producers (ISI bulletin No. IS: 8268-1976). The following are the relevant clauses from the ISI specifications for rhizobial cultures:

1) The inoculant shall be a carrier-based one
2) The inoculant shall contain a minimum of 10^8 viable cells of Rhizobium per gram of the carrier on dry-mass basis within 15 days of manufacture and 10^7 within 15 days before the expiry date marked on the packet when the inoculant is stored at 25-30°C.
3) The inoculant shall have a maximum shelf life of 6 months from the date of its manufacture.
4) The inoculant shall not have any contamination by other microorganisms.
5) The pH of the inoculant shall be between 6.0 and 7.5
6) The inoculant shall show effective nodulation on all those species/cultivars listed on the packet before the expiry date
7) The carrier material shall be in the form of powder that is, peat,

lignite, peat-soil, humus or similar materials neutralized with calcium carbonate and sterilized.

8) The manufacturers shall control the quality of broth and maintain records of tests.

9) The inoculant shall be packed in 50-75 μ low-density polyethylene packets or any other suitable container.

10) Each packet shall be marked legibly to give the following information:

    a) name of the product, specifically as Rhizobium inoculant,
    b) leguminous crop for which intended,
    c) name and address of manufacturer,
    d) type of carrier
    e) batch or code number,
    f) date of manufacture,
    g) date of expiry,
    h) net quantity meant for 0.4 ha, and
    i) storage instructions worded as follows: "Store in cool place away from direct sun and heat"

9. Precautions to be taken

- Store biofertilizer packets in cool and dry place away from direct sunlight and heat
- Use right combinations of biofertilizers
- Rhizobium is crop specific, so use in legume crop
- Do not mix with chemicals unless compatible
- While purchasing ensure that each packet is provided with necessary information like name of the product, name of the crop in which it is to be used, name and address of manufacturer, date of expiry and manufacture, batch number and instruction for use
- Use the packet before expiry, only on the specified crop, by recommended method
- Biofertilizer are live product and require care in storage
- For the best results use both N and P biofertilizers
- Use of biofertilizers is being emphasized along with chemical fertilizers and organic manures
- Biofertilizers are not replacement of fertilizers but can supplement their requirement

10. Status of biofertilizers in groundnut

Legumes can potentially fix about 80% of their own nitrogen and in addition can contribute to the yield of subsequent crops. However, the potential is seldom realized due to one or the other constraints, abiotic or biotic. In groundnut, the biological nitrogen fixation is further compounded by the promiscuity of the crop. But identification of competitive, efficiently nodulating, nitrogen fixing strains of rhizobia and their inoculation can solve the problem of ineffective nodulation by native rhizobia in groundnut. A number of bacteria have been developed over the years for inoculation in groundnut:

Table 1. Status of different categories of biofertilzers available for groundnut, developed at DGR, Junagadh or elsewhere

Organisms	Isolates	Yield and biomass improvement	Enhancement of uptake of the nutrients	Status
Bradyrhizobium, Rhizobium	IGR6, IGR40, NC92, Tt9, TNAU14, NRCG4, NRCG9, NRCG22	11-17%	N	Released
Bacillus polymyxa Pseudomonas striata Pseudomonas spp.	BM8, BM4	5-11%	P	Available
Pseudomonas fluorescens	PGPR1, PGPR2, PGPR4, combination of PGPR1, PGPR2 and PGPR4	13-23%	N, P, Fe and others	Released
Consortium of beneficial microorganisms	Compatible strains of bradyrhizobia, PGPR, PSM	11-17%	N, P, Fe and other nutrients	Released
Vesicular-Arbuscular Mycorrhizae	*Gigaspora margarita*, *Glomus mossese*, *Gigaspora scutellospora*	7-10%	P, micro-nutrients and water	Available

11. Different biofertilizers of groundnut

(a) Rhizobium

This belongs to bacteria group and is the classical example of symbiotic nitrogen fixation. The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia, which is readily utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. It has been estimated that different legume crops could fix 40-250 Kg N/ha/year by the microbial activities of *Rhizobium.*

The successful nodulation of groundnut by a strain of Rhizobium depends on its ability to overcome antagonism and competition from other soil microorganisms and other strains of rhizobia.

Excess or insufficient soil moisture reduced nitrogen fixation and a 40% reduction in light intensity considerably reduced the rate of nitrogen fixation.

Deep sowing results in the development of elongated hypocotyls and poor rooting, nodulation, and nitrogen fixation especially in the Spanish types.

Application of cobalt has been reported to increase nodulation in high pH soils and increase in the rate of nitrogen-fertilizer application decreases the rate of nitrogen fixation.

The use of various chemicals for control of pests affects nitrogen fixation. Application of seed dressing fungicides (e.g. Thiram) is detrimental to the *Rhizobium* coated onto the seed. Hence, an indirect method of *Rhizobium* application (furrow application) may be suitable under such situation. Three organophosphorus insecticides, Fensulphothion, Quinalphos and Disulfoton had no adverse affect on nodulation. Carbofuran had no affect on nodulation whereas Thimet, Heptachlor, and Dasanite reduced the number of nodules. Application of the above insecticides, however, did not reduce nitrogen fixation. Experiments conducted at the National Research Centre for Groundnut, Junagadh revealed that Thiram and Bavistin are not toxic to *Bradyrhizobium* strains, IGR 6 and IGR 40, even at 100 ppm. Therefore, these strains can safely be inoculated on groundnut seeds, pre-treated with the fungicides.

Nodulation, nodule development and nodule functioning are all reduced at low iron concentrations. Groundnuts are very susceptible to lime-induced iron deficiency. Like many soil microorganisms, bradyrhizobia can secrete siderophores to aid Fe uptake. One strain which produces siderophores, NC92, was found to nodulate groundnuts better in low Fe conditions than strain TAL 1000, which does not. Bicarbonate adversely affected nodulation and nodule functioning and effects were more pronounced on nitrogen fixing plants than on those given mineral N. Lack of response to inoculation with *Rhizobium* NC 92 at one of the two locations, out of 11 multilocations trials on groundnut in AICRPO was attributed to toxic levels of manganese.

Commonly occurring soil fungi, actinomycetes and bacteria compete with rhizobia for the limited pool of nutrients available in the rhizosphere and inhibit growth by excretion of antibiotics. Protozoans like Colpoda can drastically reduce soil *Rhizobium* titres. Therefore it is essential to develop rhizobial strains with high saprophytic competence. Competitive ability of the inoculants strains with the native rhizobial population affects the nodule occupancy and nitrogen fixation. Development of competitive strains in a highly promiscuous crop like groundnut is challenging because the native flora are generally more competitive than the inoculants strains. Several competitive strains (NRCG4 and NRCG9) of rhizobia have been identified in groundnut having multiple inhibitory effects on native flora like antibiosis, bacteriocinogeny and siderophore production.

Inoculation with effective Rhizobium strains

Many newly cleared fields lack *Rhizobium* species that nodulate groundnut. *Rhizobium* inoculation has increased yields in several field experiments in India. However, nodules formed by the native strains may not fix nitrogen or their fixation rates may often be inadequate. Lack of response to inoculation and low yield in groundnut are probably due to competition from ineffective strains in the soil. Experiments conducted by ICRISAT at Patancheru and other centers of the All-India Co-ordinated Research Project on Oilseeds (AICORPO) showed that inoculation with an effective Rhizobium strain NC92 increased the yield of groundnut cultivar 'Robut 33-1' in such fields where ineffective native strains were present.

Inoculation with NC92 also increased yield of the cultivar 'JL 24' at Junagadh in Gujarat. Two strains of *Bradyrhizobium*, IGR 6 and IGR 40 have been identified at National Research Centre for Groundnut, Junagadh. Inoculation of groundnut with IGR 6 and IGR 40 increased the groundnut yield by 11%. In Tamil Nadu, a strain of *Rhizobium* (TNAU 14) and another of *Bradyrhizobium* (Tt9) are used for commercial manufacture of inoculants. Inoculating the soil with a suspension of peat containing rhizobia in water has been found successful.

In so far as the quantum of fertilizer savings due to inoculation is concerned, a field trial by All India Co-ordinated Research Project on Biological Nitrogen Fixation (AICRP-BNF) at Coimbatore showed that inoculation of rhizobia alongwith *Pseudomonas* PS 2 at 100% N and P gave maximum pod yield and substituted for 25% N and P. Groundnut rhizobia decline after rice cultivation due to adverse effect of waterlogging, so inoculation is needed each year. In a three year study on vertisols by the AICRP-BNF, Parbhani, *Rhizobium* inoculation of groundnut grown with recommended dose of NPK (25:50:30) increased the pod yield by 3.9 q/ha while FYM alone @ 5t/ha increased it by 1.5 q/ha, combined application of FYM and *Rhizobium* increased it by 7.3 q/ha. Nodulation, N and P uptake, *Rhizobium* population in soil, etc., were all enhanced due to combined application of FYM and *Rhizobium*.

Application of sulphur @ 40 kg S/ha in combination with *Rhizobium* was found beneficial for nodulation parameters and groundnut kernel yield in a field experiment on a sandy loam. Protein and oil content were increased by S fertilization.

To overcome the problem of competition and develop highly nodulating and nitrogen fixing strains of groundnut rhizobia, a large number of groundnut rhizobia were isolated at the Directorate of Groundnut Research having multiple competitive traits like bacteriocinogeny, antibiosis (Figure 2) and siderophore (Figure 2).

Subsequently, two highly competitive and efficiently nodulating (Figure 3) nitrogen fixing strains of groundnut rhizobia, NRCG4 and NRCG9 were identified by testing in pots and field at the experimental sites of NRCG

(Figure 3; Figure 4) which outperform standard cultures like IGR6, IGR40, NC92 and TAL 1000 (Table 2). Inoculation of these strains gave higher number of nodules as compared to uninoculated control and standard cultures (Figure 5).

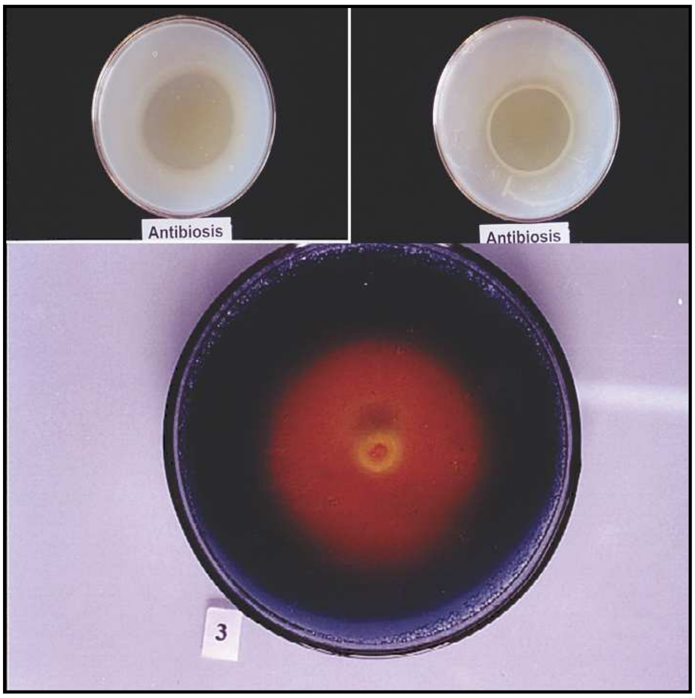


Figure 2. Production of antibiotics and siderophore by competetive strains of groundnut rhizobia

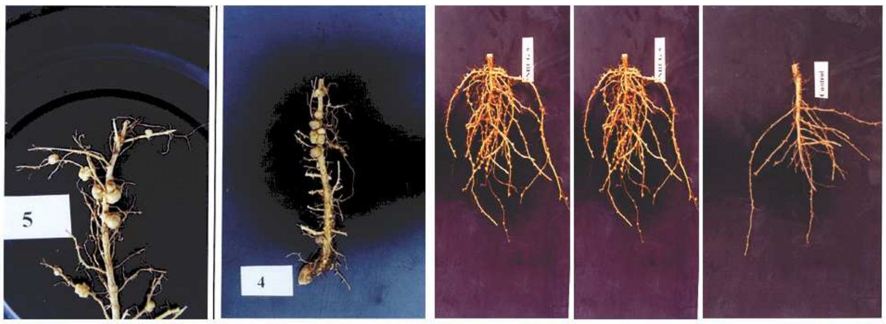


Figure 3. Effect of inoculation of competetive strains of groundnut rhizobia (NRCG4 and NRCG5) on the nodulation in groundnut (cultivar JL24) in field conditions

Figure 4. Field trial conducted with competetive strains of groundnut rhizobia (NRCG4, NRCG9, etc.) at NRCG, Junagadh

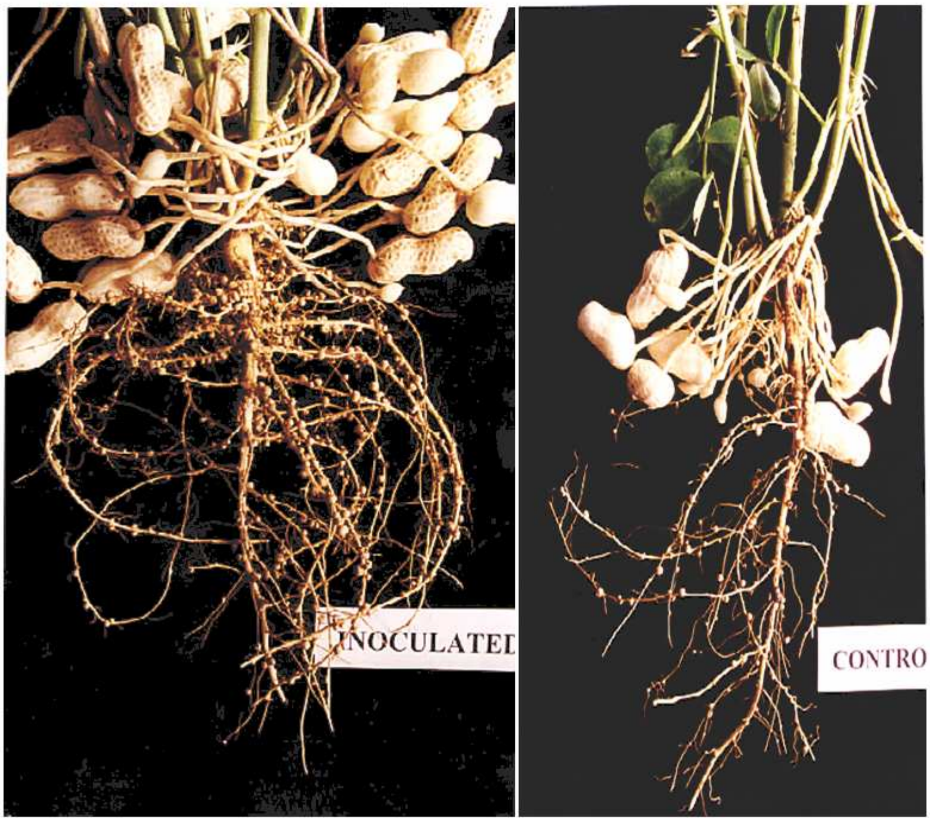


Figure 5: Effect of inoculation of competitive strain of groundnut rhizobium (NRCG4) on the nodulation in groundnut (cultivar GG2); left: inoculated; right: uninoculated

Table 2. Results of the trials conducted at NRCG in field with competitive strains of groundnut rhizobia (NRCG4 and NRCG9)

Isolate	Pod yield (kg/ha)	Haulm yield (kg/ha)	Nodule /plant	N in plant (%)	N in kernel (%)
Control	1876	2655	69	1.42	4.20
TAL1000	2035	2970	84	1.63	4.66
NC92	2020	2910	91	1.66	4.77
NRCG4	2215	3005	112	1.73	4.86
NRCG9	2195	2845	105	1.78	4.84

Subsequently, the competitive strains were tested in farmers field, in and around, Junagadh (Figure 6) and the potential of NRCG4 and NRCG9 were demonstrated (Table 3). Inoculation of NRCG4 and NRCG9 significantly improved groundnut yield over uninoculated control and standard inoculant, NC92.

Table 3. Comparison of the demonstration of competitive strains of groundnut rhizobia (NRCG4 and NRCG9) at experimental station and in farmers' field (cultivar GG2)

Isolate	Pod yield (kg/ha)					
	NRCG	Vadal (1)	Vadal (2)	Vadal (3)	Vadal (4)	Pooled mean
Control	1460	1265	1370	1578	1465	1427
NRCG4	1765	1470	1605	1870	1710	1684
NRCG9	1690	1505	1680	1805	1825	1701
NC92	1565	1425	1305	1635	1765	1539

Figure 6. Demonstration of effect of competitive strains of groundnut rhizobia (NRCG4 and NRCG9) in farmers' field

Thereafter, these two highly competitive and efficiently nitrogen fixing strains (NRCG4 and NRCG9), were tested in AICRP(G) centres for three years and found highly promising and outperformed the standard strains like NC92 and TAL1000. These two *Bradyrhizbium* strains have been recommended for application in groundnut as seed treatment.

(b) Plant growth promoting rhizobacteria (PGPR)

The mechanisms of PGPR-mediated enhancement of plant growth and yields of many crops are not yet fully understood. However, in groundnut the possible explanations (Figure 7) include:

- ability to produce ACC deaminase to reduce the level of ethylene in the roots of the developing plants thereby increasing the root length and growth
- ability to produce hormones like indole acetic acid (IAA), gibberellic acid and cytokinins
- asymbiotic nitrogen fixation
- ammonification
- help in nodulation and biological nitrogen fixation

- antagonism against soil-borne phytopathogenic fungi of groundnut like *Aspergillus niger*, *Sclerotium rolfsii* and *Aspergillus flavus* by producing siderophores, β-1,3-glucanase, chitinases, antibiotics, fluorescent pigment, volatile substances and cyanide
- solubilization of mineral phosphates
- mineralization and mobilization of other nutrients
- alleviation of biotic and abiotic stresses by modulating the functional environment of plant tissues

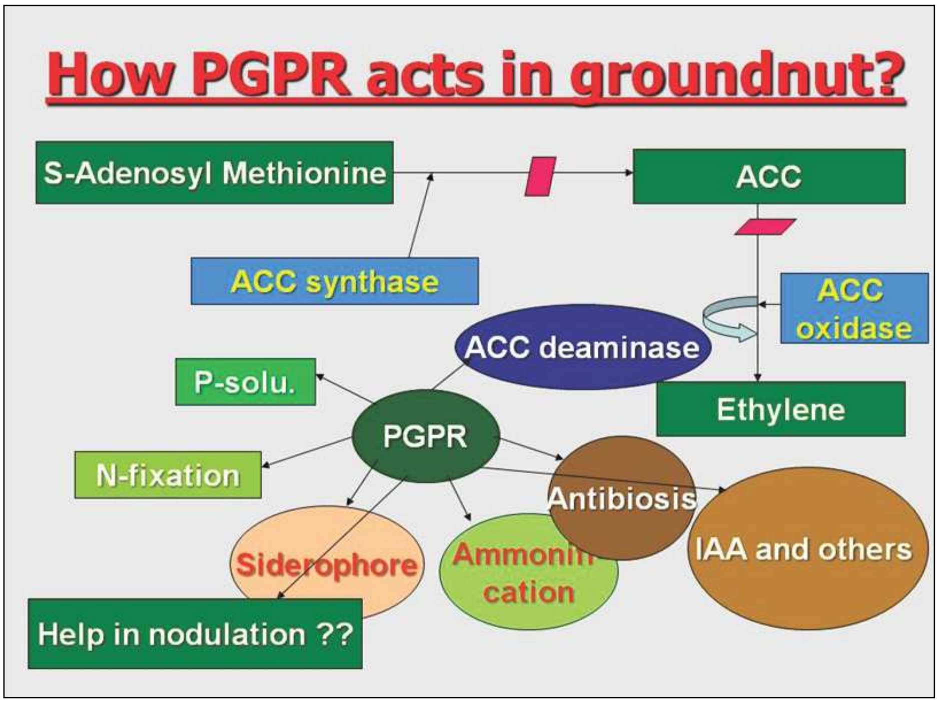
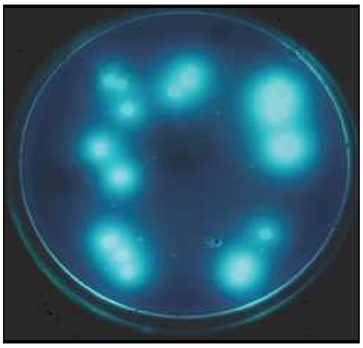


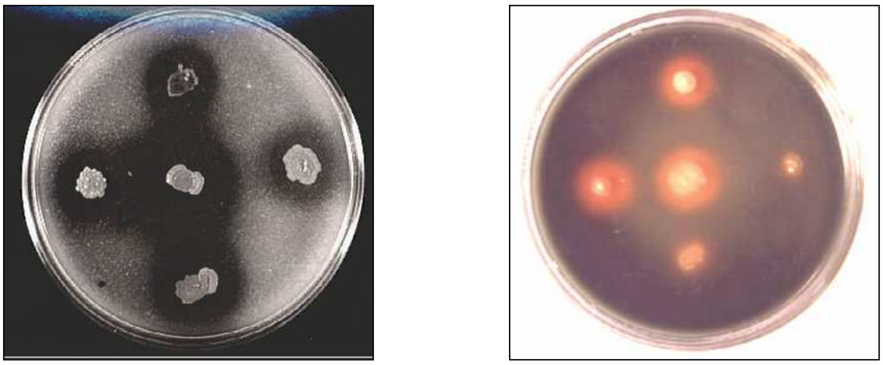
Figure 7. Possible mechanism(s) of enhancement of groundnut yield by PGPR

Efforts have been made in India in the past to develop plant growth promoting rhizobacteria suitable for groundnut under rain-fed situations. At the National Research Centre for Groundnut, Junagadh, nine different isolates of plant growth promoting rhizobacteria were selected from a pool of 233 rhizobacterial isolates obtained from the groundnut rhizosphere on the basis of ACC-deaminase activity. Subsequently, all the isolates were tested for production of other plant growth promoting traits like phosphate solubilization; nitrogen fixation; production of siderophore, antifungal metabolites (Figure 8), hormones; antifungal activities against major soil-borne fungal pathogens and other related attributes. Initial screening was also made on the basis of enhancement of seedling vigour (Figure 9), and enhancement of plant growth in pots (Figure 10). All the nine isolates were identified as *Pseudomonas* spp (Table 4). Four of these isolates viz., PGPR1, PGPR2, PGPR4 and PGPR7 (all fluorescent pseudomonads) were the best in producing siderophore and IAA in addition to characters like tri-calcium phosphate solubilization, ammonification (Table 4) and *in vitro* inhibition of *Aspergillus niger* and *Aspergillus flavus.* The performances of these selected plant growth promoting rhizobacterial isolates were evaluated for three years in the pot and field trials (Figure 11). Seed inoculation with three isolates viz., PGPR1, PGPR2 and PGPR4 resulted in significantly higher pod yield than the control, in pots, during rainy and post rainy seasons. The contents of nitrogen and phosphorus in soil, shoot and kernel were also enhanced significantly in treatments inoculated with these rhizobacterial isolates in pots during both the seasons. In the field trials, however, there was wide variation in the performance of the PGPR isolates in enhancing the growth and yield of groundnut. Seed bacterization with PGPR1, PGPR2 and PGPR4 significantly enhanced pod yield (23-26%, 24%-28%, and 18-24%, respectively), haulm yield and nodule dry weight over the control (Table 5). Seed bacterisation with PGPR1, PGPR2 and PGPR4 suppressed the soil-borne fungal diseases like collar rot of groundnut caused by *Aspergillus niger* and PGPR4 also suppressed stem rot caused by *Sclerotium rolfsii*.



Fluorescent pseudomonads

Antifungal activity against *S. rolfsii*



Phosphate solubilization Production of siderophore

Figure 8. Plant growth promoting traits exhibited by different strains of PGPR

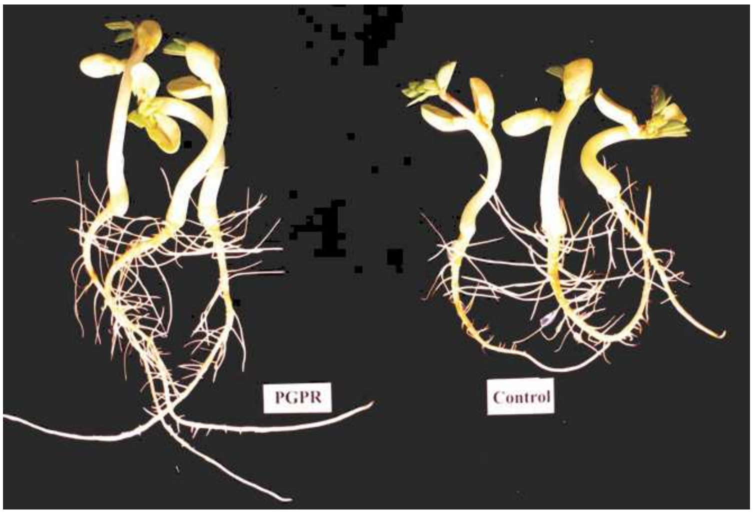


Figure 9. Screening of PGPR in laboratory condition by seedling bioassay

Figure 10. Evaluation of PGPR in pots with groundnut (cultivar GG2)

Table 4. Attributes of plant growth promoting rhizobacteria developed at NRCG (DGR), Junagadh, isolated from groundnut

Isolates	Root length (cm)	Zone of siderophore (mm)	Catechol siderophore (mg/mg pro)	IAA (mg/L)	Identity
PGPR 1	8.40	5.0	0.106	3.6	*P. fluorescens*
PGPR 2	9.10	7.6	0.121	7.8	*P. fluorescens*
PGPR 3	7.90	-	-	-	*Pseudomonas* sp.
PGPR 4	8.87	12	0.137	9.3	*P. fluorescens*
PGPR 5	8.03	4.4	0.102	-	*P. fluorescens*
PGPR 6	7.97	4.6	0.075	3.9	*P. fluorescens*
PGPR 7	9.00	9.5	0.109	11.8	*P. fluorescens*
PGPR 8	8.07	4.3	0.054	-	*Pseudomonas* sp.
PGPR 9	7.6	4.5	0.072	-	*Pseudomonas* sp.
Control	6.03	-	-	-	-

These strains of pseudomonads were also compatible with the seed treating chemicals like Carbendazim. On the basis of results obtained at on farm trials, it was proposed to evaluate these cultures in AICRP(G) centres and TAR-IVLP programme in different villages in Junagadh. The cultures were evaluated at eight different locations for three consecutive years under rain-fed situations under AICRP(G). In majority of the locations, inoculation of PGPR1, PGPR2 and PGPR4 and their combinations in the form of a consortium enhanced the growth, yield and nutrient uptake of groundnut significantly. Pooled results of 3 years revealed that seed inoculations with either PGPR1, 2 or 4 increased pod yield by 16.5 to 18.1 % over the control with cultivar JL24. These PGPR isolates have been recommended for groundnut cultivation under rain-fed situations during kharif groundnut workshop held at Bangalore from 11-13th April' 2003. This was followed by 100 odd FLDs conducted all over the country to demonstrate the inoculation effects of PGPR on growth and yield of groundnut and yield response of 18% was obtained with a B:C ratio of 2.46 (ICBR obtained was 6.4). There was significant enhancement of pod and haulm yield of groundnut under TAR-IVLP programmes taken up in different villages at Junagadh and nearby localities demonstrated over a period of time with yield advantage of 7-11%.

Table 5. Effect of inoculation of PGPR on the growth and yield of groundnut

Yield attributes observed at harvest	Pod yield (kg/ha)			Haulm yield (kg/ha)			100 seed mass (g)			Number of pod/plant			Shelling out turn (%)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	1678	1872	1938	2349	2687	2332	31.91	26.25	37.61	17.6	20.3	20.0	70.89	71.12	74.45
PGPR 1	2071	2350	1900	2899	3985	2932	34.28	34.25	36.41	22.3	25.6	19.6	72.15	73.25	74.77
PGPR2	2154	2320	1917	3016	4092	2830	36.21	33.75	36.40	22.6	25.4	18.3	71.96	74.00	74.17
PGPR3	1422	2170	2192	1991	3222	3252	31.25	37.25	38.38	16.2	22.2	25.3	70.12	74.50	73.77
PGPR4	1936	2315	2288	2710	3672	2950	37.24	35.50	36.77	20.6	24.6	24.6	73.25	74.50	73.10
PGPR5	1685	2157	2366	2359	2860	2722	36.12	35.50	37.70	18.4	22.6	24.0	70.36	75.25	74.50
PGPR6	1488	2175	2566	2083	3142	2550	37.45	35.75	36.71	15.6	21.6	27.6	69.23	75.00	73.83
PGPR7	1326	2045	2315	1856	3082	2640	33.72	34.25	36.86	15.0	21.2	24.3	71.23	73.75	74.67
PGPR8	1791	1955	2538	2686	2772	2775	34.32	35.50	37.20	18.6	20.4	26.3	72.56	75.25	73.80
PGPR9	1576	1945	1785	2679	2927	3037	34.85	32.50	37.54	16.3	21.0	18.6	69.21	73.25	74.32
LSD (P=0.05)	287	258	193	301	956	268	3.21	2.59	NS	2.45	3.21	3.78	1.87	2.57	NS

Figure 11. Field evaluation of PGPR strains in groundnut with cultivar GG2 (left: with PGPR; right: uninoculated)

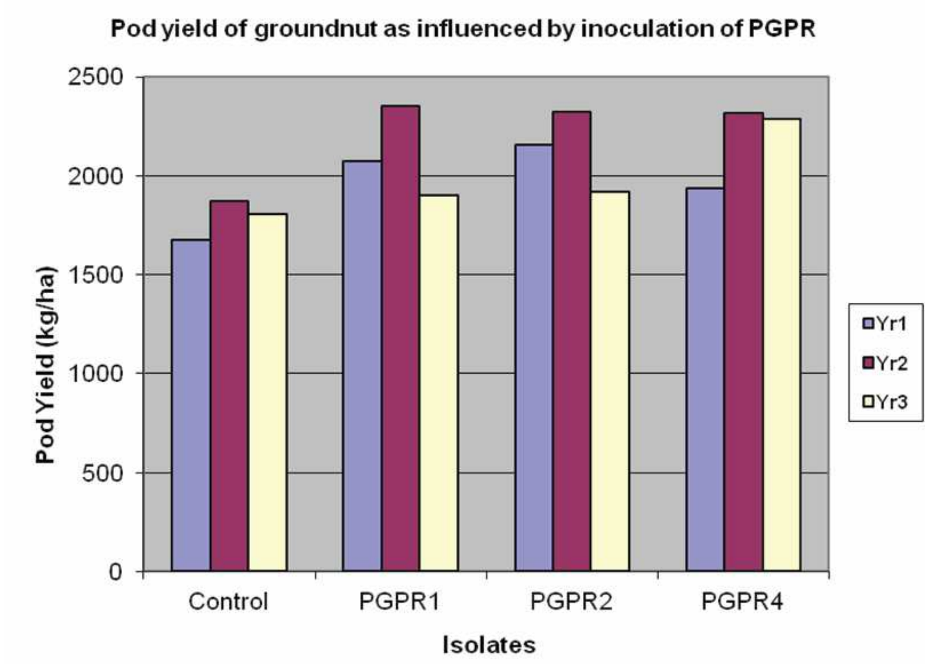


Figure 12. Effect of application of PGPR on the pod yield of groundnut

Benefits that can be harnessed using PGPR in groundnut:

- Yield enhancement of more than 10%
- Improvement in soil health
- Improvement in nutrient mobilization and uptake of P, K, N, Fe, Zn, etc.
- Produces plant growth promoting substances like IAA and iron chetating substances like siderophore
- Control of incidence of soil-borne fungal pathogens in groundnut
- Easy to apply
- Eco-friendly
- Nominal in cost
- Very fast growing and ease in large scale multiplication
- Compatible with seed treating chemicals like Bavistin (Carbendazime)/Thiram
- Can be used both for rain-fed and irrigated groundnut

(C) Consortia of beneficial bacteria

Reasons for consortia

- Single organism does not have all the properties to provide PGP traits
- There is chance of failure of inoculation of single strain as back up does not exist
- Thus, application of a mixed biofertilizer formulation comprising mutually compatible strains of PGPR, PSM and rhizobia would be best available option to provide a cascade of benefits to the plant

Development of consortia and effective delivery system

As inoculation of a single strain of microorganism may not be sustainable for providing all required benefit to plant as well as there is chance of failure, a mixed biofertilizer formulation would be best available option to provide a cascade of benefits to the plant by different organisms in mixture. The mixture would also provide an essential backup in case of failure of an organism. Thus, consortia were identified comprising mutually compatible and competent strains of phosphate solubilizing microorganisms (PSMs), groundnut-rhizobia and plant growth promoting rhizobacteria (PGPR) after pair-wise testing for compatibility. DGR has developed two such consortia (Table 6) of beneficial bacteria as biofertilizer mixture application of which can enhance the yield of groundnut by 10-20%. The consortium-I comprised of PGPR

(*Pseudomonas fluorescens* biovar V BHU1 and *Pseudomonas maculicola* S1(6)), PSB (*Pseudomonas* sp. BM8; *Bacillus polymyxa* BM4), and rhizobia (NRCG4 and NRCG9). The consortium II comprises *Pseudomonas* sp. C185 + *Pseudomonas* sp. ACC3 + *Bacillus megaterium* + *Pseudomonas* sp. ACC10 + groundnut rhizobia NRCG 22 and NRCG4 (Table 6). The consortium can take care of plant growth promotion, better phosphate solubilisation and nitrogen fixation. Thereafter, the consortia were tested at NRCG, Junagadh with significant yield improvements (Figure 13) and in AICRP(G) centres as seed treatment, and application of the consortium enhanced the yield of groundnut by 10-21% in groundnut in AICRP(G) trials and recommended for rain-fed groundnut cultivation. The population dynamics of individual members of the consortia were also determined using intrinsic antibiotic resistance patterns which indicated that majority of the members of the consortia gave sizeable population. The population of the microorganisms in the rhizosphere soil ranged from 0.02 X 10^6 to 34.0 X 10^6 cfu/g during the crop duration. The consortium has been formulated as talcum based powder and found very efficient and recommended for seed treatment for both irrigated and rain-fed situations. The benefits of consortia have been demonstrated through FLDs and on farm trials at different parts of the country.

Table 6. Compatible strains of groundnut rhizobacteria identified for development of consortia

Organisms	**Identity**	**Phenotypes**
PSM	*Bacillus polymyxa* BM4	$Sid^+MPS^+IAA^+$
	Bacillus megaterium	Sid^+MPS^+
	Pseudomonas sp. ACC10	$Sid^+MPS^+IAA^+$
	Pseudomonas sp. BM8	Sid^+MPS^+
PGPR	*Pseudomonas fluorescens* C185	$Sid^+MPS^+IAA^+ACC^+$
	Pseudomonas sp. ACC3	$Sid^+MPS^+IAA^+ACC^+Amm^+$
	Pseudomonas fluorescens BHU1	$Sid^+MPS^+IAA^+ACC^+$
	Pseudomonas fluorescens S1(6)	$Sid^+MPS^+IAA^+Afa^+$ (*A. niger*) ACC^+
Groundnut-rhizobia	NRCG 4	Sid^+IAA^+
	NC 92	Sid^+IAA^+
	NRCG22	Sid^+IAA^+
	TAL1000	Sid^+IAA^+
	NRCG9	Sid^+IAA^+

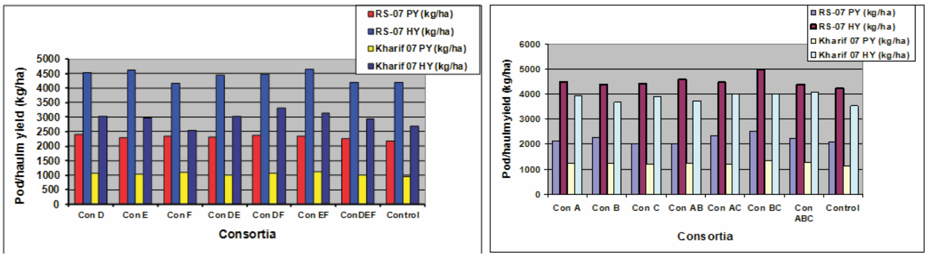


Figure 13. Enhancement in pod yield of groundnut by application of consortia of beneficial bacteria

Delivery system

Different delivery systems of a consortium of beneficial bacteria were evaluated in field trials with groundnut cultivar TG37A. The beneficial bacterial consortium consisted of PGPR (*Pseudomonas fluorescens* BHU1 and *Pseudomonas maculicola* S1(6)), PSM (*Pseudomonas* sp. BM8 and *Bacillus polymyxa* BM4) and groundnut rhizobia (NRCG 4 and NC 92). The material for carrier /delivery systems for consortium were FYM, talcum powder, kaoline, sterile farm soil, charcoal, groundnut seed, and irrigation water.

Application of the consortium as a formulation in FYM proved to be the best and resulted in 18% enhancement in pod yield over un-inoculated control (Table 7). This also resulted in increase in length of shoot and root, yield of haulm, shelling turnover and hundred kernel mass.

Table 7. Effect of application of different delivery systems of beneficial consortium comprising compatible beneficial bacteria on groundnut yield, cultivar TG 37A

Treatments	Pod yield (kg/ha)	Haulm yield (kg/ha)
Control	1329	4225
Irrigation	1380	4588
FYM	1575	5200
Charcoal	1395	4400
Kaoline	1360	4320
Soil	1530	5012
Seed	1496	4363
Talcum	1403	4560

Benefits that can be harnessed using consortia of beneficial bacteria in groundnut:

- Yield enhancement upto 20%
- Improvement in soil health
- Improvement in nutrient mobilization and uptake of P, K, N, Fe, Zn, etc.
- Enhancement in biological nitrogen fixation through use of efficiently nodulating and nitrogen fixing strains of rhizobia
- Dependence on nitrogenous fertilizer can be minimum
- Enhancement in phosphate solubilization and uptake
- Produces plant growth promoting substances like IAA and iron chetating substances like siderophore
- Strains of the consortium are compatible to each other
- Easy to apply
- Eco-friendly
- Nominal in cost
- Very fast growing and ease in large scale multiplication
- Compatible with seed treating chemicals like Bavistin (Carbendazime)/Thiram
- Can be used both for rain-fed and irrigated groundnut

(d) *Azospirillum*

It belongs to bacteria and is known to fix considerable quantity of nitrogen in range of 20-40 Kg N/ha in the rhizosphere in plants such as cereals, millets, oilseeds, cotton ,etc. The efficiency of *Azospirillum* as biofertilizers has increased because of its ability of inducing abundant roots in several plants like rice, millets and oilseeds. Considerable quantity of nitrogen fertilizer (25-30%) can be saved by the use of *Azospirillum* inoculant. *Azospirillum* cultures synthesize considerable amount of biologically active substances like vitamins, nicotinic acid, indole acetic acid, gibberellins, etc which help plants in better germination, early emergence and better root development.

(e) *Azotobacter*

It is the most important and well-known free-living nitrogen fixing aerobic bacterium. It is used as a biofertilizer for plants like rice, cotton, vegetables, oilseeds,etc. Azotobacter have been found to produce some antifungal substance which inhibits the growth of some soil fungi like *Aspergillus*.

(f) Phosphate solubilizing microorganisms (PSM)

The most important aspects of the phosphorus cycle are microbial mineralization, solubilization and mobilization, besides chemical fixation of phosphorus in soil. The mineralization of organic phosphorus, which is left over in the soil after harvesting, or added as plant or animal residues to soil, takes place through enzymatic activity of microorganisms.

Phosphate solubilizing bacteria and fungi play an important role in converting insoluble phosphatic compound such as rock phosphate, bone meal and basic slag and particularly the chemically fixed soil phosphorus into available form. These special types of microorganisms are termed phosphate solubilizing microorganisms (PSM). Such bacteria and fungi can grow in media where $Ca_3(PO4)_2$, $FePO_4$, $AlPO_4$, apatite, bonemeal, rock phosphate or similar insoluble phosphate compounds are the sole source of phosphate. Such organisms not only assimilate phosphorus but also cause a large amount of soluble phosphate to be released in excess of their own requirements. These efficient microorganisms are found to mineralize insoluble phosphates into soluble form due to enzymatic activity. The bacteria save P_2O_5 up to 30-50 kg /ha. PSM produced organic acids like malic, succinic, fumaric, citric, tartaric acid and alpha ketoglutaric acid hasten the maturity and thereby increase the ratio of grain to straw as well as the total yield.

A number of phosphate solubilizing bacteria were tested in India and found to enhance the pod yield, nodule dry weight, haulm yield and nutrient uptake significantly over control in Groundnut. *Pseudomonas striata* produced about 11% increase in groundnut yield, increasing the pod yields from 0.9 t/ha in control to 1.0 t /ha upon inoculation.

For the purpose, two promising phosphate solubilizers, both pseudomonads, PSM3 and PSM5, were identified and found to give higher pod yield, plant biomass, P content in shoots and in kernels as compared to uninoculated controls. These two cultures exhibited better performance than *Pseudomonas striata*. Inoculation with *Pseudomonas* fluorescens BM6, *Pseudomonas striata* and consortium resulted in significant increase in pod yield, maximum with the inoculation of a consortium of PSM cultures (13%). The best combination of culture x dose was consortium + 40 Kg P_2O_5 as SSP which resulted in 14% increase in pod yield.

(g) AM fungi

Some of the soil borne fungi are capable of mobilizing P /making it available from the immobile form of phosphorus by its hyphal structures. These soil microbes have mutualistic association with plants. Besides phosphorus, these fungi also mobilize zinc and sulphur.

The term mycorrhizae literally means "fungus root" to denote the association between certain soil fungi and plant roots. Mycorrhizal plants increase the surface area of the root system for better absorption of nutrients from soil especially when the soil are deficient in phosphorus.

The endomycorrhizae are known as arbuscular mycorrhizae which possess special structures known as vesicles and arbuscules, the latter helping in the transfer of nutrients from soil into root system.

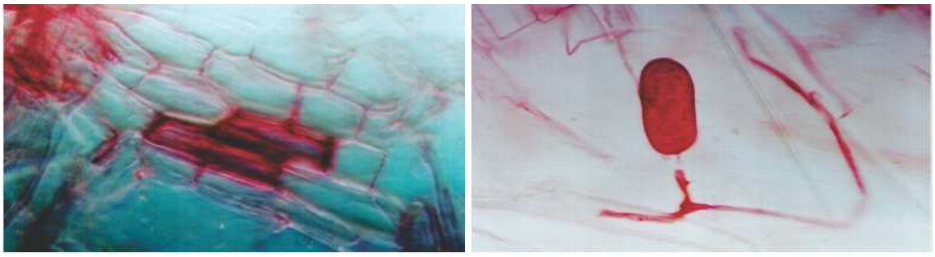
Since large scale production of AM in axenic culture is not yet attained, inocula have been produced in pot cultures or in small field plots on plants grown under carefully controlled conditions, to avoid contamination by plant pathogens. Such inocula have comprised infected roots or spores and hyphae trapped in soil, peat and clay carriers.

Mechanism of improved plant growth due to AMF application:

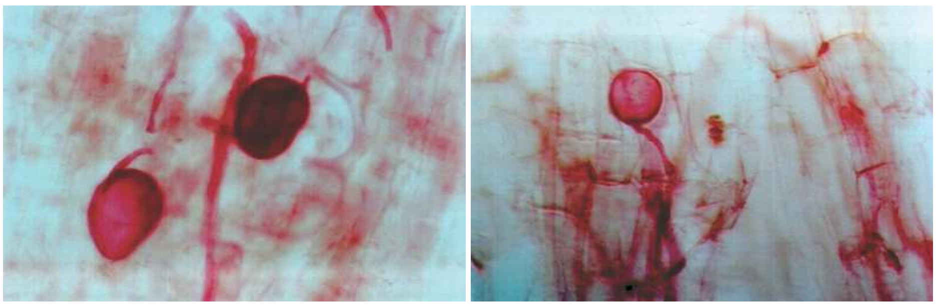
- Nutrient uptake
- Production of growth promoting substances
- Beneficial interactions between soil microorganisms
- Drought tolerance
- Disease resistance
- Nearly 25 to 50% of phosphatic fertilizers can be saved through inoculation with efficient AM fungi.

Application of AM fungi in groundnut

Arbuscular mycorrhizal fungi (AM fungi) are reported to enhance uptake of macro- (P & K) and micro-nutrients (Fe, Zn, B, Mo, etc.) in many agricultural crops besides enhancing the rhizosphere zone and mining of water under moisture-deficit stress conditions. In marginal and sub-marginal soil, AM fungi are expected to exert better performance as compared to normal soil conditions. Therefore, to enhance nutrient uptake and mobilization in groundnut, four strains of AM fungi viz., *Glomus etunicatum, Glomus fasciculatum, Glomus mosseae,* and *Gigaspora scutellospora* were tested both in pots and field conditions, to evaluate the effect. Inoculation of different AM fungi (1500-2000 chlamydospores/ 100 g of soil) had significantly better effects on groundnut growth in terms of shoot and root biomass; nodule number and mass; and pod yield of groundnut, cultivar GG 2, as compared to un-inoculated control (Table 8). There was remarkable increase in root volume and root biomass in AM fungi inoculated treatments and in some cases, the root volume increased nearly two folds (inoculation with *Glomus mosseae*). Studies on VAM root colonization (Figure 14) indicated that there was significant root colonization and formation of arbuscules and vesicles upon inoculation of the AM fungi as compared to un-inoculated control.



Glomus etunicatum



Glomus fasciculatum

Figure 14. Colonization of AM fungi in groundnut root

Table 8. Evaluation of the effect of AM fungi on yield of groundnut in field (cultivar TG 37A)

	Pod yield (kg/ha)	Haulm yield (kg/ha)
Control	2693	4727
Glomus etunicatum	2840	4583
Glomus fasciculatum	2997	5223
Gigaspora scutellospora	2950	4407
Glomus mosseae	2443	4927

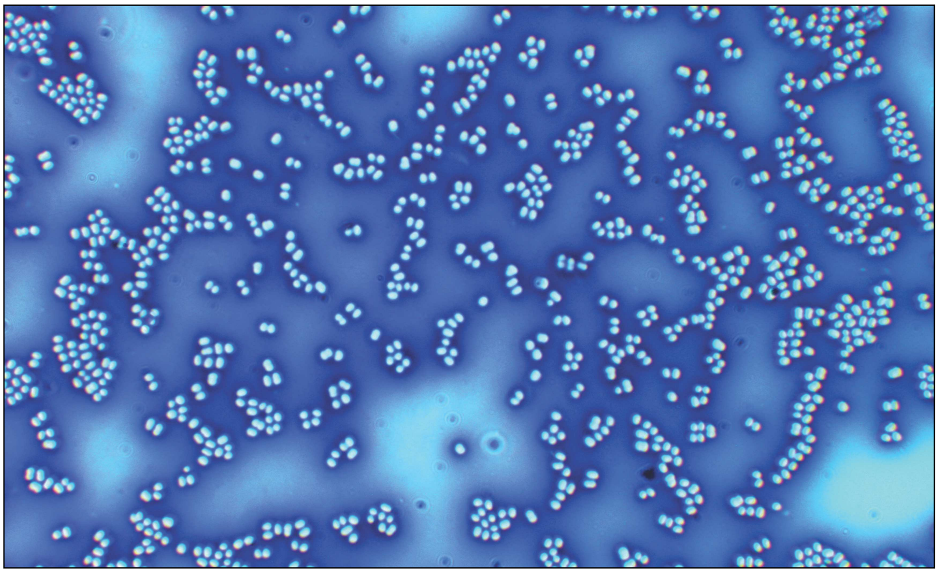
Application of AM fungi *Glomus fasciculatum* and *Gigaspora scutellospora* significantly improved the pod yield of groundnut in field conditions (11% over control) as compared to control (Table 8). Besides uptake of P and uptake of micronutrients was also improved in groundnut due to application of AM fungi.

12. Reasons for non-responsiveness of biofertilizers

- Difficult to visually perceive differences of about 10% or less
- Hidden benefit of increased proportion of nitrogen fixed from air accrue
- Beneficial responses not obtained in 1/3 rd of the trials
- Spatial variability with respect to physical properties, fertility gradients
- Not conducting experiments in the same field, no knowledge of previous history of plots, differential fertilization of previous crop affects present crop, not measuring NO_3-N to account for/understand these differences
- Mid-season drought affects inoculated plants eliminating earlier observed differences
- Improper site selection, shade effects of trees, too few replications
- Improper handling of biofertilizers & improper application
- Not measuring N yield or keeping at least cereal controls for legumes

13. Conclusions

Application of biofertilizer is a long term sustainable perspective and should not be thought for a short term gain. Application of effective and competent strains of biofertilizer can improve yield of groundnut and other crop on a sustainable basis by improving the nutrient supply, creating healthy soil environment and suppressing soil-borne pathogens. Nevertheless, the application of biofertilizer should be made with prudence.



माइक्रोस्कोप के निचे पी.जी.पी.आर.

प्रक्षेत्र मे पी.जी.पी.आर. का परीक्षण

Indofait role in groundnut yields

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