Application of DAPG-producing fluorescent pseudomonas inoculants improved groundnut yield and reduced stem- and collar-rot incidence

Conventional methods of cultivation of groundnut involve application of nitrogenous and phosphatic fertilizers along with application of significant amounts of pest control chemicals for controlling soil-borne fungal diseases and pests. High rate of incidence of soil-borne fungal pathogens particularly *Aspergillus niger*, *Aspergillus flavus*, and *Sclerotium rolfsii* during rainfed groundnut cultivation is causing great set back in total production vis-à-vis productivity. Thus, management of the pathogen is of urgent need for sustained production of groundnut in India. Development of suppressive soils containing DAPG (diacetylphoroglucinol) and phenazine antibiotic producing strains of fluorescent pseudomonads is one of the management strategies for combating the menace of these pathogens. Thus, DAPG-producing fluorescent pseudomonads were identified for using them as biofettilizer for ehnacing growth, yield and nutrient uptake of groundnut besides combating the incidence of soil-borne diseases through the development of suppressive soils.

DAPG-producing fluorescent pseudomonads as plant growth promoter and disease suppressor: Application of DAPG-producing fluorescent pseudomonads were successful in reducing the mortality seedlings from 73-82% in pathogen control to 21-42% in different treatments inoculated with the inoculant strains against natural death of 5-9% (**Figure** 1) in field conditions with susceptible cultivar GG20 during kharif 2012 to 2016. Besides, suppression of plant pathogens by making soil naturally suppressive to pathogens, growth and yield of groundnut have also been improved substantially due to plant growth promoting activity. Application of *Pseudomonas putida* DAPG 4 (pod yield: 2405-4435 kg/ha); *P. putida* DAPG2 (pod yield: 2307-3959 kg/ha); *P. putida* FP86 (pod yield: 2703-3721 kg/ha); and *Pseudomonas fluorescens* FP98 (pod yield: 2755-3750 kg/ha) improved the yield of cultivar GG20 by 13-21% over the years over un-inoculated control (pod yield: 2514-3671 kg/ha).

Fig. 1. Impact of inoculation of DAPG-producing *Pseudomonas putida* DAPG4 in suppressing stem rot of groundnut in field condition

Economic/soil/other reliable impact

- **Benefit accrued in multilocation testing**: Evaluation through AICRP(G) centres during kharif 2014-2016 recorded significant enhancement in the yield of groundnut (16-148%) in seven locations with *P. fluorescens* FP98, *P. putida* DAPG4, and *P. putida* FP86 over uninoculated control. Besides, incidence of collar and stem rot was also reduced appreciably.
- **PGPR traits:** The DAPG-producing fluorescent pseudomonads have multiple plant growth promoting (production of IAA, siderophore, phosphate solubilization, ammonification, ACC deaminase activity) traits and antifungal activities (antibiotics and HCN production).
- Besides, most of the strains are capable of solubilizing Zn, Mn, and K in soil and make them available to the plants.
- **NutGrow**: Formulation in liquid, charcoal, talcum powder and peat has shelf-life of more than one year at room temperature, compatible with Bavistin/Thiram, eco-friendly and can be applied with FYM/irrigation water/drip/drenching etc.
- **Soil health**: Improves soil health by inhibiting pathogens with yield advantage of 10-20%.
- **Availability**: '**NutGrow'** is ready for commercialization and available with ICAR-DGR.

Endophytes – a boon for groundnut cultivation in drought-affected areas

With the changing climatic scenario, it is predicted that there will be increase in frequency and intensity of stresses, particularly drought, affecting productivity. As a result, vast stretches of arable land are likely to be further added to the already existing 40% of global land under arid and semi-arid areas. As groundnut is predominantly a rainfed crop grown in semi-arid tropics, and susceptible to drought, productivity of groundnut may reduce drastically because of inadequate and uneven distribution of rainfall in different parts of country. As endophytic microorganisms are believed to have co-evolved with plants and helped them in acclimatizing and circumventing the extremities of terrestrial ecosystem during their transition from aquatic life, this group of organisms can be exploited to alleviate drought and prevent reduction in yield.

Fig. 1: Impact of endophyte on pod yield of groundnut without any supplementary irrigation

Application of endophytic bacteria like *Bacillus firmus* J22, *Bacillus subtilis* REN51, and *Pseudomonas pseudoalcaligenes* SEN29, identified in groundnut prevented the loss of yield of groundnut by 15-25% as compared to uninoculated control, with the application of a single irrigation at the time of sowing, and no irrigation thereafter with cultivar TG37A during successive summer seasons (Figure 1). With single supplementary irrigation at 55 DAE, the application of these endophytes were able to prevent yield loss to the tune of 17-25% over control.

Economic/soil/other reliable impact

- **Water saving**: Summer groundnut is raised with the application of 10-12 irrigations depending upon soil type. But with the application of drought-stress alleviating endophytic bacteria, it was found that at least 4-5 irrigations can be saved without drastic reduction in pod yield.
- **Horizontal expansion in area**: the saved water can be utilized for horizontal expansion of irrigated area and thus total production can be improved substantially.
- **Economic benefit**: As application of three protective irrigations after emergence with endophytes like *Pseudomonas pseudoalcaligenes* SEN29 and *Bacillus firmus* J22 gave 2260-2309 kg/ha of pod yield equivalent to that obtained with 10 irrigations after emergence (2233-2483 kg/ha), doubling the area with same available water can double the production and income.
- **Impact in farmers' field**: Demonstration in the farmer's field at Anantapur District of Andhra Pradesh with cultivar Kadiri 9 and 180 mm rainfall and two protective irrigations gave 32% yield improvement with *Bacillus firmus* J22 over untreated control.

Endophytes are saviour for groundnut in drought-prone areas like Anantapur, the largest groundnut growing district in the country with occurrence of drought frequently.

- **Availability of cultures:** available with ICAR-DGR for commercialization.
- **Shelf-**life: Bacilli are endospore-forming and has indefinite shelf-life in formulation and are compatible with seed treating chemicals of groundnut.

Endophytes – a savior of cultivation of groundnut in salt affected areas

With the changing climatic scenario, it is predicted that the sea level is likely to rise and sea water ingression in coastal areas will render a vast area unfit for cultivation. Repeated use of ground water will also increase the level of salinity. In Gujarat around 1.5 lakh ha of groundnut area is affected by salinity in Kutch, Porbandar, and Mangrol areas. It is also predicted that around 50000 ha of land will add to salinity-affected areas in India. It is also known that salinity badly affected yield of groundnut. Thus, to make groundnut cultivation profitable in salinity affected areas in India, Bacillus firmus J22, an endophytic bacterium which has been reported repeatedly to alleviate salinity stress, has been demonstrated as FLD in the farmers field.

Fig. 1: Demonstration of endophytes in farmers field at Bhuj, Kutch, Gujarat

Application of endophytic bacteria like *Bacillus firmus* J22, and *Bacillus subtilis* REN51, identified in groundnut prevented the loss of yield 15-20% as compared to uninoculated control in soil with EC level of 5-6 at Bhuj in experimental plots with susceptible cultivar TG37A. In summer 2017, 16 FLDs and in Kharif 2017, 30 FLDs were conducted in farmers field to demonstrate the beneficial effects of this endophyte.

Economic/soil/other reliable impact

Yield enhancement in salinity affected areas of Kutch (Kharif 2017): Demonstration was laid in 16 farmers field with plot area of 0.1 ha with soil salinity of 4.5 to 6.2 EC witj cultivar GG2. The pod yield in control plot ranged from 1000 to 3800 kg/ha with a mean of 2058 kg/ha whereas in treated plot, it ranged from 1250 to 4400 kg/ha with a mean value of 2427 kg/ha. The average increase in pod yield due to seed treatment with *Bacillus firmus* J22 over the control was 17.89%. The haulm yield increased by 5.65% due to seed treatment over the control.

Economic benefit in kharif groundnut: The bacterial seed treatment was economically beneficial as a net return of Rs 79,320/ha along with BCR of 2.37 was recorded under treated plot as against Rs 63,895/ha with BCR of 2.15 in control.

Demonstration Summer 2017 in salinity affected areas in Kutch: The pod yield in control plot ranged from1634 to 3064 kg/ha with a mean of 2246 kg/ha whereas in treated plot, it ranged from 2010 to 3403 kg/ha with a mean value of 2685 kg/ha. The average increase in pod yield due to seed treatment with bacteria over the control was 19.52%. The haulm yield increased by 5.92% due to seed treatment over the control. The pod yield increase was mainly due to increased number and weight of pods per plant under the treated plot as compared to the control.

Economic benefit in summer groundnut: The bacterial seed treatment was economically beneficial as a net return of Rs 63, 306/ha along with BCR of 2.28 was recorded under treated plot as against Rs 49,281/ha with BCR of 2.05 in control.

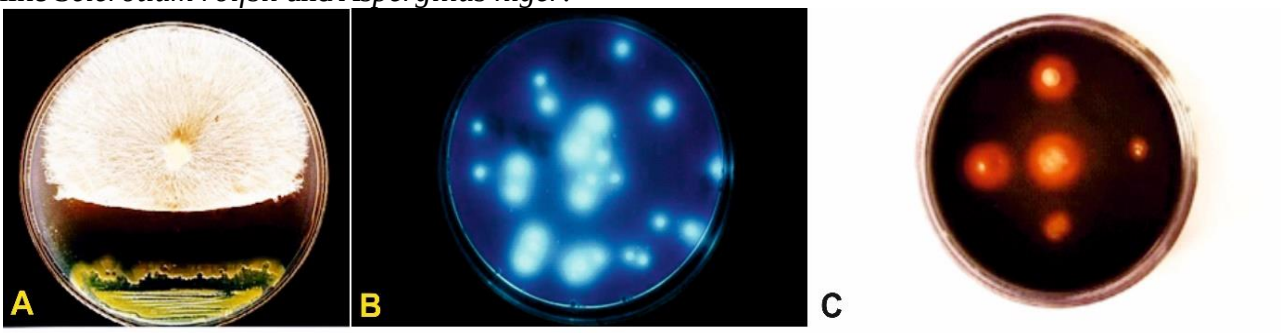
Availability of cultures: available with ICAR-DGR for commercialization.

Shelf-life: Bacilli are endospore-forming and has indefinite shelf-life in formulation and are compatible with seed treating chemicals of groundnut.

Possibility of expansion in groundnut area: Application of endophytes become saviour of groundnut cultivation in salinity affected areas and more salinity affected areas can be brought under cultivation with the help of endophytes in future.

NutBoost: A PGPR Formulation Enhances Groundnut Growth and Yield

Beneficial soil microorganisms play a very important role in sustainable crop production. These microbes are helpful in biogeochemical recycling of mineral nutrients as well as improving soil health and fertility. The formulation of the consortium of Plant Growth Promoting Rhizobacteria (PGPRs) comprising of *Pseudomonas gessardii* BHU1 (PGPR1), *Pseudomonas putida* S1(6) (PGPR2), and *Pseudomonas* sp. BM6 (PGPR4), called '**NutBoost'** was originally isolated at Junagadh from groundnut rhizosphere. These microorganisms are having multiple plant-growth promoting attributes like ACC deaminase, phosphate solubilization, and production of siderophore, antifungal metabolites, hormones, and ammonification traits. Besides, these PGPR isolates also exhibited solubilization of Zn and potassium (K) and exhibited antifungal activities against major soil-borne fungal pathogens like *Sclerotium rolfsii* and *Aspergillus niger*.



Traits of PGPR of 'NutBoost' formulation: A= antifungal activity against *S. rolfsii*; B= production of fluorescent pigments; C= production of siderophore

Application of these organisms have been found to enhance growth, yield, and nutrient uptake in groundnut at Junagadh, in farmers' fields, at multi-locations in India, tested through AICRP(G) centres for three years and demonstration through FLDs. Application of PGPR1, 2 or 4 alone or in combinations can enhance pod yields by 16.5 to 18.1 % over the un-inoculated control. These PGPR isolates have been recommended for irrigated and rainfed groundnut cultivation in India on the basis of the performance. The FLDs conducted in India with groundnut showed yield response of 18% with B:C ratio of 2.46 (ICBR: 6.4). Subsequently, a liquid formulation of PGPR1, PGPR2 and PGPR4 (compatible to each other) was developed and named "**NutBoost**" with a shelf-life of more than one year at room temperature.

Economic/soil/other reliable impact

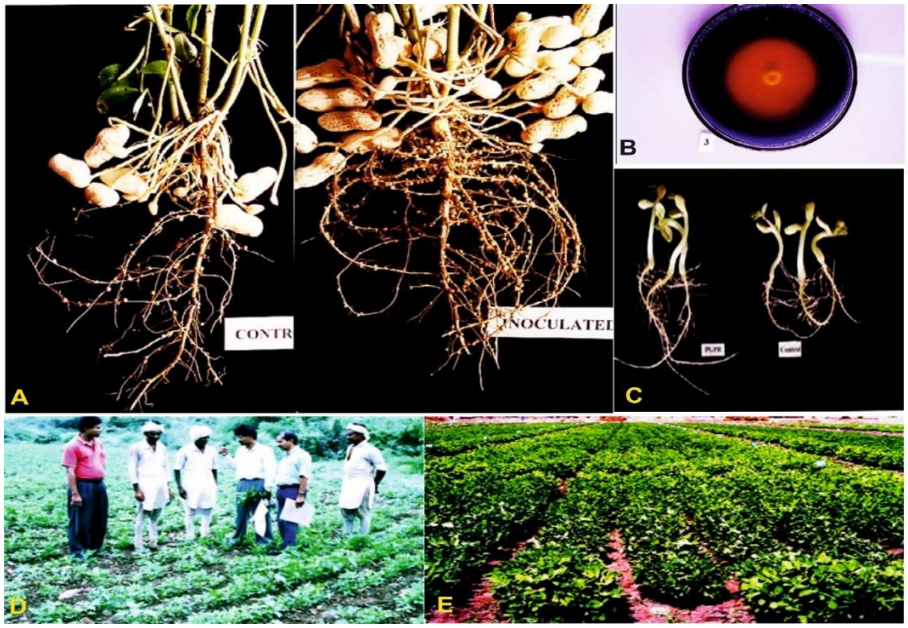
- Improvement in soil health with yield advantage of at least 10%.
- Improvement in nutrient mobilization and uptake like P, K, N, Fe, Zn, etc. (P, Zn, and K solubilizers) and useful for both irrigated and rainfed conditions
- Produces plant growth promoting substances like IAA and iron chelating substances like siderophore and compatible with seed treating chemicals like Bavistin (Carbendazime)/Thiram
- Control of incidence of soil-borne fungal diseases in groundnut (stem and collar rot)
- These mutually compatible strains are very fast growing and ease in large-scale multiplication through co-culturing
- Formulated "**NutBoost"** (liquid/charcoal/talcum powder/FYM) has a shelf-life of more than one year at room temperature and can be applied easily through irrigation water or through FYM or as seed treatment

Availability of the formulation:

The technology is ready for commercialization and the details are available with AgrInnovate India Limited and with the ICAR-Directorate of Groundnut Research, Junagadh.

'NutMagic' Bacterial Formulation Augments Groundnut Growth and Yield

Soil microorganisms play a very important role in sustaining crop production by nutrient recycling, mineralization and processes by which plants can thrive and agriculture can prosper. Accordingly, **'NutMagic',** a formulation of mutually compatible strains of PGPR ((*Pseudomonas gessardii* BHU1, *Pseudomonas putida* S1(6)); PSB (*Enterobacter cloacae* BM8; *Bacillus polymyxa*), and rhizobia (*Sinorhizobium americanum* NRCG4 and *Rhizobium* sp. NRCG9) has been developed application of which can improve soil health, nutrient mobilization and uptake and enhance yield upto 20% both in irrigated and rainfed groundnut cultivation.



Traits and effects of constituents of NutMagic formulation: A=enhanced nodulation, B= siderophore production, C=root growth on inoculation, D=demonstration in farmers' field. E=station trails

The consortium can take care of plant growth promotion by ACC deaminase activity, siderophore production, ammonification, suppression of pathogens, and solubilization of Zn and K; better phosphate solubilisation by PSBs; and improved biological nitrogen fixation by rhizobia. The consortium has been recommended after testing at Junagadh and at multilocations through AICRP(G). These bacteria of the consortium are also compatible with seed treating chemicals. The benefits of consortia have been demonstrated through FLDs and on farm trials at different parts of the country.

Economic/soil/other reliable impact

- These ecofriendly and mutually compatible strains improves in soil health, improves yield upto 20% and compatible with seed treating chemicals like Bavistin (Carbendazime)/Thiram.
- Improvement in nutrient mobilization and uptake like P, K, N, Fe, Zn, etc. (P, K, and Zn solubilizers) and useful for both irrigated and rainfed conditions
- Enhances biological nitrogen fixation and produces plant growth promoting substances like IAA and iron chetating substances like siderophore
- Dependence on nitrogenous and phosphatic fertilizer can be reduced by 50%.
- Very fast growing and ease in large- scale multiplication as co-culturing reduces time.
- Can be used both for rain-fed and irrigated groundnut
- Formulated "**NutMagic"** (liquid/charcoal/talcum powder/FYM) has a shelf-life of more than one year at room temperature and can be applied easily through irrigation water or through FYM or as seed treatment

Availability of the formulation:

The technology is ready for commercialization and the details are available with AgrInnovate India Limited and with the ICAR-Directorate of Groundnut Research, Junagadh.