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

Situation Analysis:

Groundnut is a major oilseed crop in India with an average production of around 7.0 million tonnes. It contributes nearly 30% of the domestic oilseed production. Conventional methods of cultivation 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. Being a leguminous crop, the nitrogen requirement of the crop is met largely through biological nitrogen fixation; however, phosphorus requirement is important for the crop. Phosphorus and potassium solubilizing pseudomonas bacterial inoculants play an important role in nutrient mobilization to groundnut crop. High rate of incidence of soil-borne fungal pathogens during rainfed groundnut cultivation is causing great set back in total production vis-à-vis productivity. A number of soil-borne fungal pathogens viz. *Aspergillus niger*, *Aspergillus flavus*, and *Sclerotium rolfsii* cause devastating havoc to groundnut in most of the major groundnut growing states. Thus, management of the pathogen is of urgent need for sustained production of groundnut in India. Besides application of cultural practices, development of resistant cultivars, application of fungicides and biocontrol agents, development of suppressive soils containing DAPG (diacetylphoroglucinol) and phenazine antibiotic producing strains of fluorescent pseudomonads (**Figure 1**) is one of the management strategies for combating the menace of these pathogens.

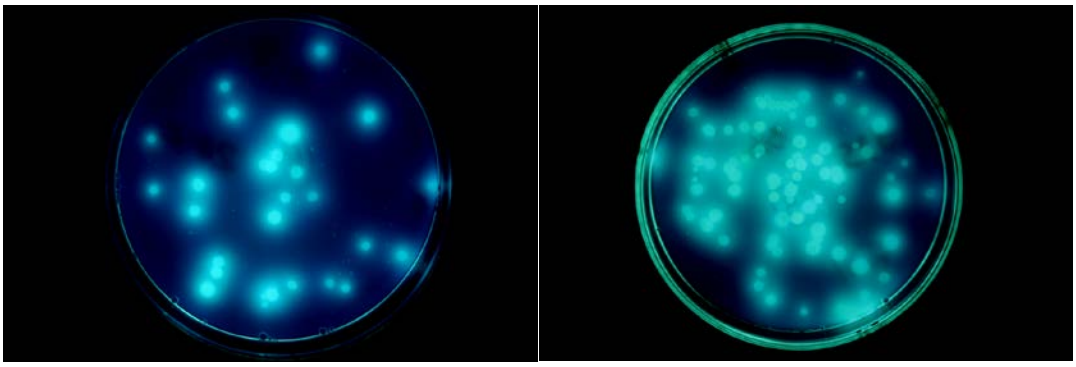

Figure 1. Colonies of DAPG-producing fluorescent pseudomonads under UV light

Thus, development of natural suppressive soil by enhancing the population of DAPG-producing strains of fluorescent pseudomonads might be one of the best options to reduce the inoculum load of the pathogen below threshold level so that pathogenicity does not outbreak. At the same time, this group of fluorescent pseudomonads has multiple plant growth promoting traits for enhancing growth, yield and nutrient uptake. Thus, use of these DAPG-producing rhizobacterial isolates has great potential as biofertilizer additionally having biocontrol traits.

DAPG-producing fluorescent pseudomonads as plant growth promoter and disease suppressor:

Stem rot causing pathogen, *S. rolfsii*, can infect the groundnut plant from seedling stage (**Figure 2**) to plants up to 60 days of old. However, inoculation 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** 3) in field conditions with susceptible cultivar GG20 during kharif 2012 to 2016.

Figure 2. Colonization of S. rolfsii onto cotyledon of germinating seedling of groundnut

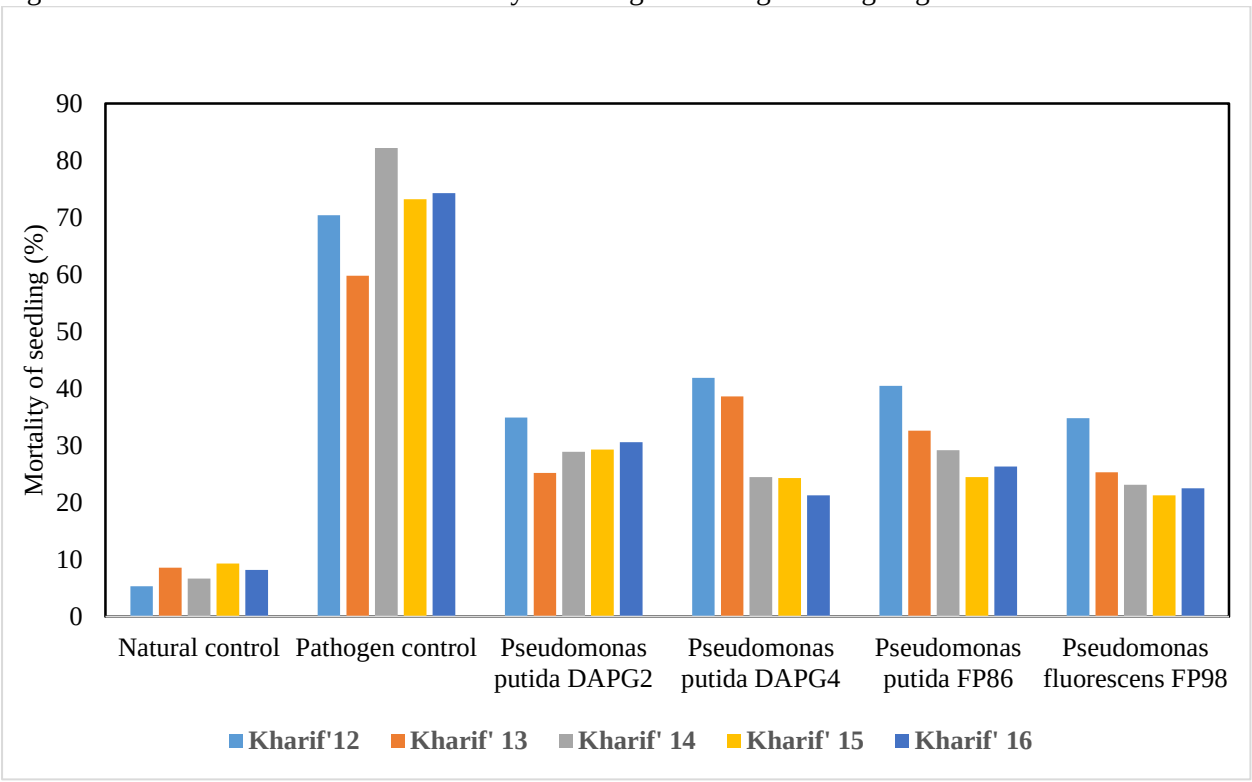

Figure 3. Impact of inoculation of DAPG-producing pseudomonads on seedling mortality

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) (Figure 4). Besides, incidence of stem rot was also reduced significantly (**Figure** 5).

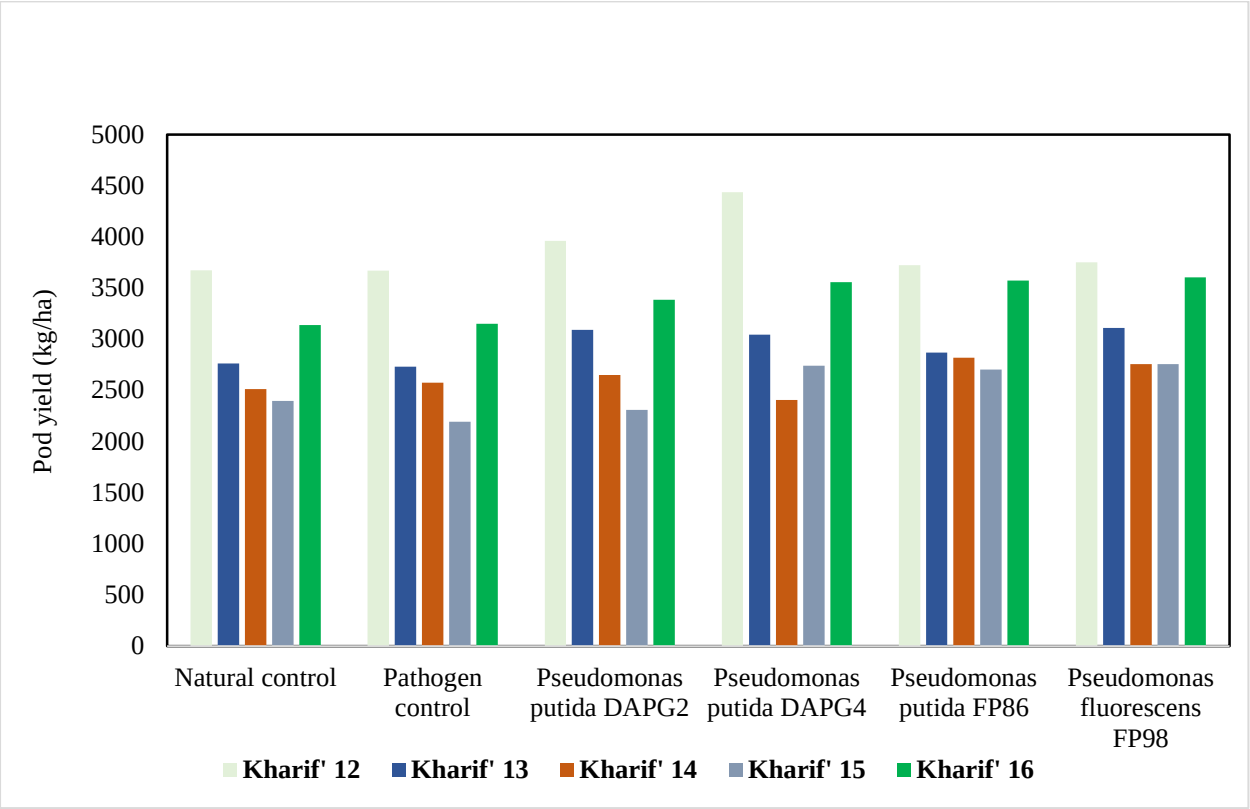

Figure 4. Impact of inoculation of DAPG-producing fluorescent pseudomonads on yield of groundnut

Figure 5. Impact of inoculation of DAPG-producing Pseudomonas putida DAPG4 in suppressing stem rot of groundnut in field condition

Performance of DAPG-producing fluorescent pseudomonads in AICRPG-trials:

DAPG-producing fluorescent pseudomonads, were evaluation through AICRP(G) centres during kharif 2014-2016 and recorded significant enhancement in the yield of groundnut (16-148%) in seven locations. Significantly higher pod yield of 2460 kg/ha was achieved with the application of *P. fluorescens* FP98 (Table 1) at Shirgaon as compared to control (1896 kg/ha). Similarly, significantly higher pod yield (3192 kg/ha, 3502 kg/ha, 1689 kg/ha, 1488 kg/ha, and 1539 kg/ha, respectively) was achieved with the application of *P. putida* DAPG4 as compared to uninoculated control (2354 kg/ha, 1407 kg/ha, 1065 kg/ha, 774 kg/ha, and 1064 kg/ha, respectively) at Junagadh (JAU), Jagtial, Jalgaon, Chintamani, and Tirupati, respectively. At Bhubaneshwar and Mohanpur, significantly higher pod yield was achieved with the application of *P. putida* FP86 (2631 kg/ha and 2111 kg/ha as compared to 1856 kg/ha and 1758 kg/ha in uninoculated control, respectively). Besides, incidence of collar and stem

rot was also reduced appreciably. The trend was also similar during kharif 2015 (Table 2) and kharif 2016.

Table 1. Impact of inoculation of DAPG-producing fluorescent pseudomonads in enhancing yield of groundnut at different AICRP-G centres during kharif 2014

Centre	Best treatment	Pod yield (kg/ha)		% Increase over control	B:C ratio
		Treated	Control		
Shirgaon	*P. fluorescens* FP98	2460	1896	29.7	1.60
Bhubaneshwar	*P. putida* FP86	2631	1856	41.7	-
Junagadh (JAU)	*P. putida* DAPG4	3192	2354	35.6	-
Jagtial	*P. putida* DAPG4	3502	1407	148.9	3.07
Jalgaon	*P. putida* DAPG4	1689	1065	58.6	1.69
Tirupati	*P. putida* DAPG4	1539	1064	44.6	-
Mohanpur	*P. putida* FP86	2111	1758	20.0	2.09

Table 2. Impact of inoculation of DAPG-producing fluorescent pseudomonads in enhancing yield of groundnut at different AICRP-G centres during kharif 2015

Name of Centre	Best treatment	Pod yield Kg/ha		% increase over control	B:C ratio
		Treated	Control		
Bhubaneswar	*P. putida* FP 86	2056	1658	24	-
Durgapura	*P. putida* FP 86	5580	4769	17	4.22
Jalgaon	*P. putida* DAPG4	2437	1680	45	2.36
Junagadh	*P. putida* DAPG4	2474	1832	35	-
Mohanpur	*P. putida* FP86	2226	1886	18	3.17
Shirgaon	*P. fluorescens* FP98	2043	1761	16	1.49
Tirupati	*P. putida* DAPG4	2848	2334	22	NS

After three years of trials conducted at different AICRP(G) centres, recommendation was made to use DAPG-producing fluorescent pseudomonads for groundnut cultivation in India (**Table** 3).
Therefore, application of DAPG-producing fluorescent pseudomonads will be ideal in improving yield and managing soil-borne fungal pathogens of groundnut in India.

Table 3. Recommendations for use of DAPG-producing fluorescent pseudomonads for yield enhancement and disease suppression at different agroclimatic zones of India

Centre/Zone	Recommended DAPG-producing fluorescent pseudomonads
Bhubneswar (E & SE coastal plain zone of Odisha)	*P. putida* FP86, *P. fluorescens* FP 98 and *P. putida* DAPG 4 either as seed treatment or furrow application with FYM multiplied cultures for higher yield and disease suppression.
Durgapura (Zone-1)	*P. putida* FP86 or *P. fluorescens* FP 98 or *P. putida* DAPG 4 or P. putida DAPG2 as seed treatment for higher yield and disease suppression.
Dharwad (Northern transitional zone of Karnataka) (Zone-VIII)	*P. putida* FP86 or *P. fluorescens* FP 98 or *P. putida* DAPG 4 or P. putida DAPG2 as seed treatment for higher yield and disease suppression.
Jalgaon (Plateau and assured rainfall zone of Maharashtra)	*P. putida* DAPG 4 as seed treatment for higher yield and disease suppression.
Junagadh (South Saurashtra agro climatic zone Gujarat) (Zone VII)	*P. putida* DAPG 4 as seed treatment for higher yield and disease suppression as seed treatment.
Kadiri	*P. putida* FP86 or *P. putida* DAPG 2 for enhancing groundnut yield, nutrient use efficiency and bio-control of soil borne diseases as seed treatment.
Shirgaon (South Konkan Coastal zone of Maharashtra)	*P. fluorescens* FP98 for higher yield and suppression of soil borne diseases (color rot/stem rot) as seed treatment.

About DAPG-producing fluorescent pseudomonads of groundnut:
The DAPG-producing fluorescent pseudomonads isolated from groundnut rhizosphere have multiple plant growth promoting (production of IAA, siderophore, phosphate solubilization, ammonification, ACC deaminase activity) traits (**Table** 4) 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.

Table 4. Traits of DAPG-producing fluorescent pseudomonads

Isolate	Identified as	Traits
DAPG 2	***P. putida* DAPG2**	**Afu, IAA, Ammo, Phos., Sid, HCN, etc.**
DAPG 4	***P. putida* DAPG4**	**Afu, ACC, IAA, Ammo, Phos., Sid, etc.**
FP98	***P. fluorescens* FP98**	**Afu, ACC, IAA, Ammo, Phos., Sid, Gibb., etc.**
FP86	***Pseudomonas putida***	**Afu, ACC, IAA, Phos., Sid, etc.**

Availability of culture formulation:

Liquid formulation of DAPG-producing fluorescent pseudomonads has been made, called '**NutGrow'** and is ready for commercialization by entrepreneurs.