



## INTERVENTION OF MULTITASKING PGPR IN PULSE PRODUCTION UNDER STRESS CONDITIONS

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### ABSTRACT

Pulse production in India is subjected to different environmental stresses. Moreover, in order to feed the world, increasing production without causing harm to the environment has become the central concept of the sustainable agriculture. The intensive use of chemicals pollutes the environment and calls the attention of the researchers to figure out alternatives for pulse cultivation in sustainable manner. Utilization of plant growth promoting rhizobacteria (PGPR) in pulse production has been reported to be beneficial not only in maintaining the optimum yield but also in cutting down the cost of cultivation. Some of the PGP traits considered especially for pulse production under stress conditions are nitrogen fixation, phosphorus solubilisation, sulphur oxidation, production of enzymes such as ACC-deaminase and IAA-like substances, exopolysaccharides production and bioprotection.

### Introduction

Modern agriculture is characterized by “more food production from shrinking per capita land” to meet the food demands of the rising population which is projected to be 8.5 billion by 2030, 9.7 billion in 2050 and 11.2 billion in 2100, according to a new UN DESA report, 2015. Moreover, another attention is being called for the idea of sustainability, in order to carry out the so called ‘safe cultivation’ without jeopardising the environment. During the year 2015-2016, India is proud to be the largest producer (17-18 mt), consumer (22-23 mt) and importer (4-5 mt) of pulses. Within the national boundary, Madhya Pradesh has largest production followed by Maharashtra, Rajasthan, Uttar Pradesh, Andhra Pradesh and Karnataka, together contributing about 79% of area under cultivation and 80% of pulses production. However, extensive use of chemicals in maintaining the proper nutrition of pulses creates enormous problems including environmental pollution and health hazards. Therefore, alternative options are

being suggested and opted that encompasses utilization of beneficial bacteria known as ‘Plant growth promoting rhizobacteria (PGPR).

Inoculation of beneficial bacteria with plant growth promoting properties is not new as farmers used to mix soils from a previous legume crop and soils where non legumes were grown. Plant growth promoting rhizobacteria have multifaceted properties that aids in plant growth and development (Parray et al., 2016). In this inoculation technology, several PGPR have been isolated and processed as biofertilizers, biostimulant, biopesticides etc.

Relative to the bulk soil, rhizospheric soil is inhabited by numerous and diverse PGPR due to the enrichment of rhizosphere with nutrients and chemicals such as organic acids, plant growth regulators, vitamins, amino acids and sugars found in root exudates, secretions, mucilages and mucigels. Among several microbes, bacteria have been studied in more significant manner because of its predominance and diversity.

### Plant growth promoting traits

The mechanisms of plant growth promotion through inoculation of PGPR can be classified into direct and indirect mechanisms (Fig. 1).

#### Nitrogen fixation

In pulse production, nitrogen is essential for formation of amino acids, nucleic acids, enzymes and chlorophyll, thus required in photosynthesis. Despite of the abundance of nitrogen in atmosphere (78%), the gaseous nitrogen is not available for direct assimilation by the plants. However, commercial nitrogen fertilizers are available for enhancing the productivity. But, it might be associated with economic, environment and energy issues, if mishandled and improperly used. Biological nitrogen fixation is a process in which atmospheric nitrogen is converted into available form for plants through a series of reactions involving prokaryotes and the host plant using some specific enzyme systems. Legumes are capable for biological nitrogen fixation, thus reducing the nitrogen requirement from external sources such as commercial fertilizers. *Rhizobium* is considered to be most studied bacteria as efficient colonizers in soil with the host plant. Even though BNF is an energy expensive process, it is found to be the sole process through which the unavailable atmospheric N is converted to plant usable N form, thus contributing a crucial role in nitrogen cycle. Some of the examples of plant growth promoting rhizobacteria that are efficient nitrogen fixers are *Bacillus*, *Pseudomonas*, *Erwinia*, *Caulobacter*, *Serratia*, *Arthrobacter*, *Micrococcus*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Hyphomicrobium*, *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium*.

Nitrification is another chief part of nitrogen cycle through which the inorganic ammonia is converted to nitrite and then to nitrate by nitrifying bacteria such as *Nitrosolobus*, *Nitrosomonas*, *Nitrospira* and *Nitrobacter*. The nitrification products thus released are subjected to different losses such as denitrification and leaching. These losses reduce the nutrient use efficiency of applied nitrogenous fertilizers by 45-50%. Therefore, in order to enhance the nitrogen use efficiency, nitrification inhibitors are commonly used that reduces the rate of nitrification or inhibit the process. Some plants also release secondary metabolites such as phenolic acids and flavonoids that are capable of inhibiting nitrification. However, they are found to be non-toxic to the microbial community in soil.

### Phosphate solubilization

Phosphorus is the second crucial essential element after nitrogen in terms of quantitative plant requirement. But the problem lies in the fixation of phosphorus in soil which forms insoluble complexes that are unavailable to plants. The availability of phosphorus in soil is pH dependent. Even though P is found abundant in the soil, under acidic conditions P forms insoluble phosphates with iron and aluminum while under high pH with calcium. Therefore, the insufficiency of the plant available P fraction and the concentration in the soil solution may affect the nutrition and metabolism of the plant, thus getting a setback in growth and development of the plant. Phosphate solubilizing bacteria (PSB) are those bacteria which transform the insoluble phosphorus into soluble available forms through acidification, chelation, exchange reactions, and polymeric substances formation. The utilization of PSB in legume cultivation not only reduce the high cost involved in applying phosphatic fertilizers but also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied, thus increasing phosphorus use efficiency. PSB when inoculated in soil increases its population in rhizosphere and can establish an intimate relationship with other PGPR through additive or synergistic effect which is important for improving overall performance of pulses in different soils and different cultivation practices. Some examples of PSBs found in soil are *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Klebsiella oxytoca*, etc. Fig. 2 depicts the circular genome map of *Pseudomonas* sp.

#### Sulphur oxidation

Sulphur oxidizing bacteria are those bacteria that oxidize elemental sulphur to plant available sulphate. In pulse production, sulphur nutrition is particularly important as it takes essential role in synthesis of proteins, vitamins and enzymes. Due to the widespread deficiency of sulphur in the soil, it has been given importance since recent years having its importance in pulse nutrition. *Thiobacilli* is widely studied for its ability to oxidize elemental sulphur, thus improving soil fertility. Moreover, the acidity produced through oxidation aids in solubilization of plant nutrients and improving alkali soils. Some other sulphur oxidizers are *Paracoccus denitrificans*, *Xanthobacter tagetidis*, *Thiosphaera pantotroph*, *Thiobacillus thioparus*, *T. neapolitanus*, *T. denitrificans* and *Thiomicrospira thyasirae*.

**ACC-deaminase enzyme production**

Huge area of land under pulse production in India is subjected to drought, heat stress, alkalinity and acidity which bring down its yield. Under these stress conditions, pulse crops tend to produce ethylene that reduces the growth of the plant and ultimately affects the overall yield. Many PGPR are reported to produce enzymes that inhibit the production of ethylene, that are known as ACC-deaminase producers. ACC (1-aminocyclopropane-1-carboxylate) is the immediate precursor of ethylene. The theory behind ACC-deaminase enzyme activity is to cleave ACC into ammonia and  $\alpha$ -ketobutyrate, thus restricting the formation of ethylene. The by-products of the cleaving are utilized as effective nitrogen and energy sources by those bacteria producing ACC-deaminase enzyme. Examples of ACC-deaminase enzyme producers are *Pseudomonas putida*, *Mesorhizobium loti*, *Mesorhizobium opportunistum* and *Achromobacter piechaudii*.

**IAA-like substances production**

Among auxins, Indole acetic acid (IAA) is one of the most physiologically active phytohormones. IAA takes important role in cell elongation through modifications of certain conditions such as increasing osmotic contents of cell, permeability of water into cell, decreasing cell wall pressure, enhancing cell wall synthesis and inducing specific RXA and protein synthesis. It also enhances embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting. PGPR produces IAA or IAA-like substances to influence the physiological processes of the host for their own benefits. Due to the rich supply of substrates, some PGPR are able to release IAA as secondary metabolites. It is especially important in those areas such as acidic soil where plant root growth is restricted. Some examples of IAA producers are *Enterobacter Cloacae*, *Pseudomonas savastanoi* pv. *Savastanoi* and *Azospirillum brasilense*.

**Exopolysaccharide (EPS) production**

Exopolysaccharides are polymers produced by certain microorganisms as a by-product of their metabolism. It finds its importance in pulse production especially in dry areas and soil with unfavorable structure. Exopolysaccharides have gluing properties that helps in formation of good soil structure. The improvement in soil structure enhances soil aeration and water holding capacity. Therefore, inoculation with PGPR with exopolysaccharide production ability will help in

maintaining optimum water regime in soil and improves plant growth. Other important functions of EPS are bacterial cell aggregation, cohesion of biofilms, nutrient source, conferring resistance to infection, sorption of organic compounds and inorganic ions, binding of enzymes, export of cell components and acting as sink for excess energy. Some of the beneficial bacteria that produce EPS are *Leuconostoc mesenteroides*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* etc.

**Siderophore production**

Siderophores are defined as relatively low molecular weight complexes that act as ferric chelating agents. Iron reacts with oxygen to form relatively less soluble oxyhydroxide. Bacterial siderophores react with these complexes and release iron from the insoluble complex. The toxicity of heavy metals in soil destructs the membrane bound ferric reductase enzyme and decreases iron uptake by plants showing in form of interveinal chlorosis in plant leaves. So, inoculation of PGPR with siderophore producing ability can prevent iron deficiency under this environmental stress condition. Rhizobactin is a siderophore produced by *Rhizobium meliloti* in pulse crop and it is an amino poly (carboxylic acid) with ethylenediaminedicarboxyl and hydroxycarboxyl moieties as iron chelating groups. *Paracoccus denitrificans*, *Rhizobium meliloti* and *Staphylococcus hyicus* are few examples.

**Bioprotection**

Non-pathogenic rhizobacteria with PGP traits can also enhance the plant resistance to both the biotic and abiotic stress factors. The so called 'bioprotection' can also increase the tolerance against several abiotic stresses like acidity, drought, flooding, salinity and heavy metal toxicity.

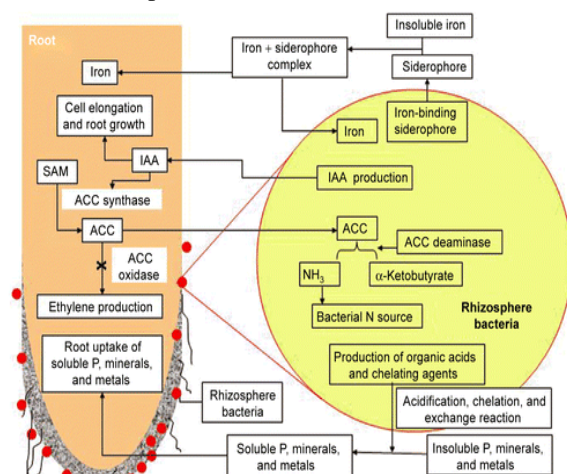
**Microbial consortium**

With these plant growth promoting traits, microbial consortium are formulated with specific purposes and utility. Microbial consortium is the mixture of beneficial microbes with different plant growth promoting activities, which acts more efficiently in consortium mode than that of the individual treatment. However, screening of the native bacterial isolates for PGP traits and checking their compatibility is the first and foremost important step in developing a microbial consortium.

**Conclusion**

PGPR are now regarded as an important tool in sustainable agriculture. In pulse production, it may serve several purposes and improve the yield of the

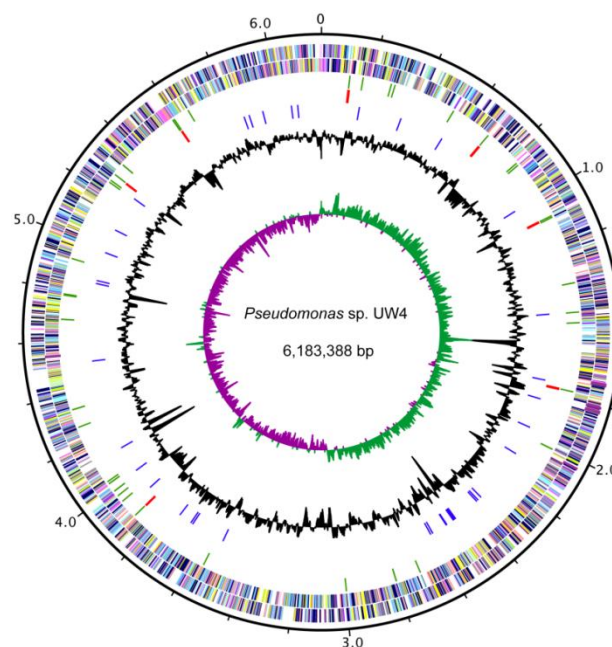
pulses without causing harm to the environment. The utilization of PGPR in pulse production will not only cut down the cost of cultivation but also help to maintain a good soil health. However, the microbial inoculants must be thoroughly checked for their effectiveness in field conditions after being formulated under in-vitro conditions. The interactions between the introduced inoculants and the native microbes must be studied carefully. Moreover, the tripartite interaction between host plant-introduced inoculants and native microbe must be examined and investigated in different cultivation practices and soil conditions. With all these information in view, PGPR finds its chief role in cultivation of pulse under stress conditions.



**Fig. 1.** Plant growth promoting (PGP) traits of the PGPR in relation to pulse production.

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**Fig. 2.** Circular genome map of *Pseudomonas sp.* From the outside in, the outer black circle shows the scale line in Mb; circles 2 and 3 represent the coding region with the colors of the COG categories; circle 4 and 5 show tRNA (green) and rRNA (red), respectively; circle 6 displays the IS elements (blue); circle 7 shows the genomic islands (orange); circle 8 represents mean centered G+C content (bars facing outside-above mean, bars facing inside-below mean); circle 9 shows GC skew  $(G-C)/(G+C)$ . GC content and GC skew were calculated using a 10-kb window in steps of 200 bp.

#### References

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