

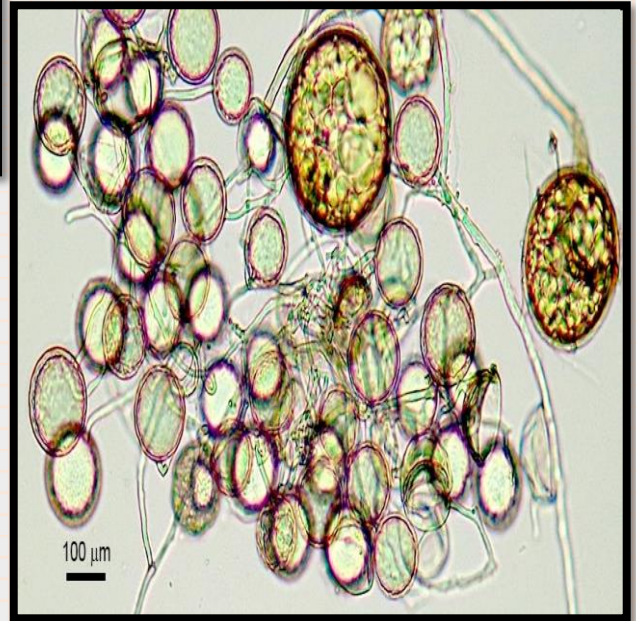
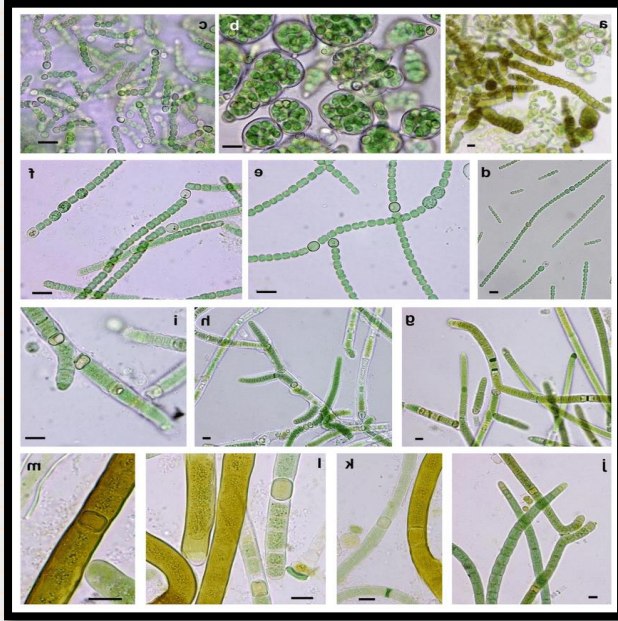
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From the desk of Chief Editor.....

Plants nutrients are essential for the production of crops and healthy food for the world's ever increasing population. Soil management strategies today are mainly dependent on inorganic chemical-based fertilizers, which cause a serious threat to human health and the environment. Bio-fertilizer has been identified as an alternative for increasing soil fertility and crop production in sustainable farming. The exploitation of beneficial microbes as bio-fertilizers has become of paramount importance in agricultural sector due to their potential role in food safety and sustainable crop production.

Bio-fertilizer can be an important component of integrated nutrients management. Microorganisms that are commonly used as bio-fertilizer components include; nitrogen fixers (N-fixer), potassium and phosphorus solubilizers, growth promoting rhizobacteria (PGPRs), endo and ecto mycorrhizal fungi, cyanobacteria and other useful microscopic organisms. The use of bio-fertilizers leads to improved nutrients and water uptake, plant growth and plant tolerance to abiotic and biotic factors. These potential biological fertilizers would play a key role in productivity and sustainability of soil and also in protecting the environment as eco-friendly and cost effective inputs for the farmers.

I wish to the edition will be helpful to researchers, Scientists, administrators and farmers / industrialists and others to understand the importance of biofertilisers for sustainable agriculture.

Gagnesh Sharma
Chief Editor

Perspectives of Plant- Beneficiary Rhizobacteria in the Era of Sustainable Agriculture

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Abstract

The world is facing the challenge of low crop production and the continuously growing human population now a days. Changing climatic conditions, excess use of chemical inputs and the contrary influence of biotic-abiotic stress conditions limit the crop productivity and soil health. In this scenario, plant growth promoting rhizobacteria (PGPR) provide an opportunity to improve the soil condition by providing the nutrition without input of chemicals and being tolerant to various stressful conditions, they also play relevant role in stress agriculture. These plant beneficiary rhizobacteria commonly known as PGPR are characterized on the basis of their traits in improving plant health like diazotrophs, phosphate solubilising bacteria (PSB), potassium solubilizing bacteria (KSB), zinc solubilizing bacteria (ZSB), production of ACC-deaminase enzyme, siderophore and phytohormones and antagonistic activity etc. Currently, a range of biotic-abiotic stresses such as salinity, drought, heat, cold and accumulation of heavy metals not only affect the agricultural productivity but also deteriorate human health. To combat all these problems, PGPR-based microbial formulations are emerging as promising agents for plant growth, biocontrol, and biotic-abiotic stress management and hence, providing their contribution in sustainable agriculture.

1.0 Introduction

The excess use of chemical inputs in the last century via, green evolution led to an increase in the yield exponentially **which comprises mainly the use of chemical inputs (pesticides, herbicides, and chemical fertilizers) and improved crop plants (through targeted breeding and advanced genetic manipulations) to** feed the population worldwide. With excessive use of chemical inputs, the overall effects were leaching out, polluting water reservoirs, destroying soil beneficial micro-organisms and eco-friendly insects and thus, making the crop more susceptible to the diseases. To combat these problems, the application of microbial inoculants like PGPR has been

since opted as a very good alternate. These microorganisms associated with plants exhibit plant beneficiary traits and carry out essential functions for their growth and development. The use of microbial based eco-friendly agricultural inputs has a long history, beginning with broad-scale rhizobial inoculation of legumes in the early 20th century and recently, the strains of various PGPR like *Bacillus*, *Pseudomonas*, *Azotobacter*, *Enterobacter* etc. have been commercialized on the large scale. With beneficial plant-microbe interactions, PGPR-based microbial formulations and biofertilizers have also been developed for the use in sustainable agriculture depending on their mode of action and effect on plant growth. In recent years, more attention has

been given to PGPR use as a biofertilizer alternative to chemical fertilizers. Apart from this, they are also characterized as stress-busters or alleviators as they are very helpful in promoting crop yield in environmental conditions under different stress regimes like salinity, drought, heavy metal and high temperature. The practical use of efficient PGPR provides an eco-friendly alternate to the chemical inputs in maintaining soil health and fertility and creating a sustainable agriculture. Hence, this article focused on various characteristics of PGPR and their implementation in agriculture so that their use may be exploited in the agriculture sector for a healthy and environment friendly upcoming for life on this earth.

2.0 Plant Growth Promoting Rhizobacteria (PGPR)

The rhizosphere is the area of soil that is directly influenced by plant root secretions and associated soil microorganisms. The rhizosphere is one of the most composite ecosystems on the earth and the vicinity is influenced by plant roots which acts as hot spot for various microorganisms. The rhizosphere serves as a habitat for several microorganisms including bacteria, archaea, fungi, algae, viruses, oomycetes, nematodes, arthropods and protozoa. These different microbial communities residing closely to the plant roots constitute rhizomicrobiome and exhibit significant effect on plant growth and development. Moreover, among them, the bacterial population residing in the vicinity of the plant roots and impart plant beneficial characteristics which are helpful in plant growth and development are refereed as plant growth promoting rhizobacteria (PGPR). Plant beneficial rhizobacteria consist a wide range of diversity which not only improve their growth but also protect them from phyto-pathogens by possessing different defence mechanisms including biopesticides and rhizomediators. PGPRs are known to stimulate plant growth either by direct or indirect mechanisms. Direct mechanisms

comprise the secretion of substances like phytohormones including auxins, cytokinins, gibberellins, ethylene and abscisic acids (ABA), liberation of nutrients and stimulation of induced systemic resistance. Many rhizobacteria play a vital role in direct mechanism, for example, Phosphate solubilizing bacteria (PSB) viz. *Bacillus*, *Agrobacterium*, *Pseudomonas* & *Dyadobacter* etc. & nitrogen-fixation as diazotrophs viz., Rhizobia group, *Azospirillum*, *Azotobacter*, *Stenotrophomonas* and AMF (Kloepper et al. 2004; Suyal et al. 2014; Kumar et al. 2019). Besides, indirect mechanisms stimulate symbiotic relationships, root growth enhancement and biocontrol characteristics by their antagonistic abilities. The rhizobacteria that can also produce siderophores could compete for iron with soil borne pathogens. Siderophore-producing rhizobacteria suppress fungal pathogens by making iron unavailable for fungal growth and also enhance availability of P to the plants through the solubilization of iron-bound phosphorus in the soil. Besides that, PGPR release several metabolites including siderophores, antibiotics like 2, 4-diacetylphloroglucinol, hydrogen cyanide (HCN), phenazine, pyoluteorin, pyrrolnitrin, and effective cell-wall degrading enzymes such as chitinases, cellulases and proteases that inhibit the growth and action of phytopathogens. Furthermore, numerous unknown mechanisms are also involved in beneficial plant microbial interactions which are responsible for plant growth and crop production and possess an important role in agriculture.

2.1 Phosphate solubilizing bacteria (PSB)

Phosphorus is an essential mineral for plant growth because it improves plant growth at cellular level and stimulates crop growth and productivity. Phosphorus has been reported to stimulate root growth, essential in seed germination, and occurs in large quantity in plant seed and fruit. Most of the soils are poor in available phosphate hence, plants with phosphate deficiency show under

developed growth, delayed maturity, wilting of leaves and ultimately reduced crop yield. Chemical fertilizers contain a large portion of soluble inorganic phosphates and after application in the soil they get rapidly fixed into the forms which are unable to utilize by the plants. Microorganisms play an important role as inhabitants of the soil in mediating the availability of soil P to the plants. Several bacterial species are responsible for the conversion of insoluble inorganic phosphates into soluble forms belong to genera like *Achromobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Flavobacterium*, *Gluconoacetobacter*, *Micrococcus*, *Pseudomonas*, *Ralstonia* and *Rhizobium* etc. (Baig 2010). Among them, *Bacillus* and *Pseudomonas* the most efficient phosphate-solubilizers isolated from diversity of crops and solubilize different phosphate substrates (Jha et al. 2009). In addition to inorganic phosphate solubilization, microbes also play an important role in organic phosphate solubilization in the soil. P-solubilizers have been reported to produce organic acids like gluconic, 2-ketogluconic, glycolic, oxalic, lactic, acetic, formic, malonic, maleic and succinic during phosphate solubilization (Chen et al. 2006). Moreover, phosphatase and phytase produced by microorganisms also cause mineralization of organic phosphates during this process (Gulati et al. 2007). Biological mechanisms of microbial P-solubilization represent novel solution for making P from the unavailable P. Phosphate solubilizing bacteria have the potential to enhance dissolution of indigenous sources of rock phosphorus and transform them into more agronomically effective P-fertilizers.

2.2 Nitrogen-Fixing bacteria

Nitrogen (N) is an element essential for the support of all forms of life. It is found in amino acids and proteins and many other organic compounds are derived from nitrogen fixation process. Biological nitrogen fixation is mainly carried out only by bacteria, which may be symbiotic or free living in nature or rhizobacteria. Nitrogen-fixing organisms are

generally active in plant root zone soil. Plants that are capable of releasing exudates exhibit higher nitrogen fixation activity in soil. N_2 must be chemically reduced to the equivalent of ammonia before it can be incorporated into biological molecules. Many rhizobacteria possess the characteristics of nitrogen fixation. Rhizobia is known for their ability to persuade nodules on the roots of leguminous plants. Within these nodules, the differentiated bacteroid helps in fixing atmospheric nitrogen into the ammonia. *Azospirillum* species belong to the facultative endophytic diazotrophs groups and exist in association with plant roots and fix atmospheric nitrogen. *Azotobacter* is an obligate aerobe, having free living characteristic in the soil and considered as most common nitrogen fixer for the crops. *Gluconobacter diazotrophicus* grow in a wide range of conditions like low pH, high sucrose and high saline conditions. It grows mainly in close association with sugarcane plants and is an efficient nitrogen fixing bacterium among endophytes. These nitrogen-fixing bacteria along with fixing atmospheric nitrogen and making it available to the plants also known to have various plant growth promoting activities as mentioned in section 2.0 and considered as potential PGPR.

2.3 Potassium solubilizing bacteria (KSB)

Potassium (K) is another major constituent within all living cells and considered as essential nutrient. Generally, soils contain K in larger amounts than any other nutrients; however most of the K is unavailable for plant uptake. Many rhizobacteria are well known for their characteristic as potassium solubilizing bacteria (KSB) which can solubilize K-containing minerals and convert the insoluble K to soluble forms available to plant uptake. Some of the rhizobacteria including *Paenibacillus* spp., *Bacillus mucilaginosus*, *B. edaphicus*, and *B. circulans* exhibit ability to solubilize K minerals such as biotite, feldspar, illite, muscovite, orthoclase, and mica. KSB are inhabitants of soils as well as plant

rhizosphere, although their population, diversity and efficiency of solubilizing K vary depending upon the soil and climatic conditions. KSB can dissolve silicate minerals and release K through the production of organic and inorganic acids, acidolysis, polysaccharides, complexolysis, chelation, and exchange reactions (Etesami et al. 2017). Hence, the large scale production and management of KSB can be an effective alternative to harmful potash fertilizers.

2.4 Zinc solubilizing bacteria (ZSB)

Zinc (Zn) is a key micronutrient, required by all living forms including plants, humans, and microorganisms for their growth and development. Zinc is a crucial micronutrient for plants and plays important role in their life cycle. Zn deficiency in the soil is one of the major micronutrient deficiencies which result into decreased crop production. Mostly, agricultural soil contains zinc in a fixed form which is unavailable to plants and thus making the soil Zn-deficient. Therefore, to solve the above problem, rhizobacteria have been emerged as an alternative to improve zinc solubilization so as to make the micronutrient available to the plants with many other plant growth promoting properties. ZSB secrete different organic acids that solubilize the fixed form of zinc to available form, which enhances plant growth promotion, yield, and soil health and fertility (Kumar et al. 2019).

2.5 ACC-deaminase production by PGPR

PGPR are the beneficial free-living soil bacteria which execute mechanisms of plant growth promotion along with capability to colonize the rhizosphere. Crop productivity may affect due to stressful regimes of climatic conditions like drought, nutrient deficiency, salinity, radiation, temperature, toxic metals and organic contaminants, water logging and various phytopathogens. Phytohormone ethylene mediates the expression of stress-encoding proteins responsible for protection of plants from the harmful effects of biotic-abiotic stresses.

Ethylene with low concentration is responsible for seedling emergence, root hair development and root extension; however its higher concentration inhibits plant growth by reducing plant root-shoot elongation, proliferation and declining other growth parameters. Many PGPR have been reported to possess production of an enzyme 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, which reduces harmful effect of ethylene in the plants. ACC-deaminase reduces ethylene level by hydrolyzing ACC to α -ketobutyrate and ammonia which is an immediate precursor of ethylene (Hontzeaset al. 2005). ACC-deaminase enzyme encoding ACC-deaminase structural gene (AcdS) have been reported in a wide range of rhizobacteria although, the expression of putative FAcdS gene may vary from one microbe to another (Singh and Jha 2016). PGPR showing ACC-deaminase activity exhibit tolerance against biotic-abiotic stresses regimes and also possess induced systemic tolerance in the host plants. Thus, PGPR retaining ACC-deaminase activity play an important role in enhancing crop yield under stressful climatic conditions.

3.0 PGPR as stress-busters in agriculture and their role in bioremediation

PGPR show multiple plant growth promoting activities and considered as an important tool for improving crop productivity. They also make their contributions in maintaining crop growth and promotion by reducing plant stress in environmental conditions like drought, salinity, and heavy metal toxicity, through decreasing ethylene level by ACC-deaminase production, increasing mineral solubilization, producing phytohormones and repressing soil borne pathogens by producing hydrogen cyanide, siderophores and antibiotics (Jaryal & Soni 2021). The environmental stress conditions such as acidity/alkalinity, temperature, desiccation and salinity exert effect on plant-microbial interaction, root colonization and the effectiveness of the microbial inoculants in

crop productivity. Phytohormone-producing heavy metal-tolerant PGPR can be effective to induce resistance against heavy metal toxicity in plants and play role in bioremediation. Some examples of heavy metal-tolerant PGPR which can be used in bioremediation are *Ochrobactrum*, *Bradyrhizobium*, *Chryseobacterium*, *Klebsiella*, *Serratia*, *Bacillus* spp., *Ralstonia*, *Exiguobacterium*, *Stenotrophomonas*, *Morganella* and *Providencia*, *Enterobacter*, *Leifsonia*, *Burkholderia phytofirmans* PsJn, *Burkholderia cepacia*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* (Nazil et al. 2020). The use of stress tolerant PGPR and understanding of their mechanisms in alleviating stressful environmental conditions can be an attractive technology in stress agriculture.

4.0 PGPR as Biofertilizers

Soil microorganisms and PGPR are important component of microbial diversity system as they play crucial role in integrated nutrient management and in maintaining the plant. Experiencing the adverse effects of synthetic input dependent agriculture, organic manures and biofertilizers are viable option for farmers to increase productivity without causing deleterious effects. Biofertilizer as a substance that contains living microbes or rhizobacteria which, when applied to seed, plant surfaces or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers contribute to the nutrition of plants through a variety of mechanisms including direct effects on nutrient availability (e.g. N_2 -fixation by diazotrophs and P mobilization by many microorganisms), enhancement of root growth (i.e. through phytohormone production), as antagonists of root pathogens or as saprophytes that decompose soil detritus, as stress alleviators and subsequently increase nutrient

availability through mineralization and microbial turnover (Fig 1).

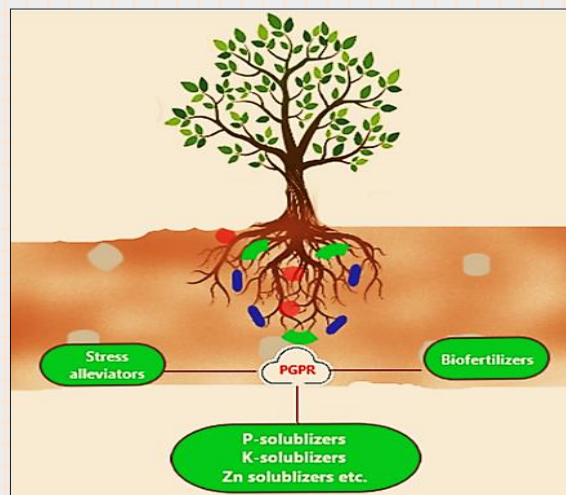


Fig.1 Multifaceted roles of PGPR

Biofertilizers are generally defined as carrier-based formulations of active or latent strains of PGPR which, directly or indirectly stimulate microbial activity and thereby increase mobilization of nutrients from soil. PGPR generally belong to genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Enterobacter*, *Pseudomonas* and *Rhizobia* etc. These PGPR-based microbial formulations possessing one or multiple plant beneficiary traits along also contribute in improving biotic-abiotic stresses on different crops and possess environmental friendly chemical-free alternative for sustainable agriculture. However, successful accomplishment of these formulations is dependent on various factors like shelf- life, variable efficacy in different agro-climatic conditions and specificity for different plants species along with soil characteristics. Some examples of PGPR-based biofertilizers exhibiting beneficial role in different crops under various biotic-abiotic stress conditions is depicted in Table 1.

Table 1. PGPR-based biofertilizers expedient for different crops under stress conditions

SI No.	PGPR strain (s)	Physiological role	Tested crops	References
1.	<i>Bacillus subtilis</i> , <i>Pseudomonas fluorescens</i> DR11, <i>Pseudomonas migulae</i> DR35, <i>Enterobacter hormaechei</i> DR16, <i>Achromobacter piechaudii</i> ARV8, <i>Azospirillum brasilense</i> , <i>Paenibacillus polymyxa</i> and <i>Rhizobium</i>	Offer drought stress tolerance	Wheat, Maize, Foxtail millet, Bean, Tomato and Pepper	[13-19]
2.	<i>Bacillus pumilus</i> , <i>Bacillus megaterium</i> , <i>Exiguobacterium oxidotolerans</i> , <i>Azospirillum</i> sp., <i>Enterobacter</i> sp. PR14 and <i>Achromobacter piechaudii</i>	Offer salinity stress tolerance	Maize, Brahmi, Tomato, Lettuce, Rice, Finger Millets and Sorghum	[20-24]
3.	<i>Bacillus amyloliquefaciens</i> , <i>Paenibacillus xylandedens</i> , <i>Streptomyces</i> sp., <i>Pseudomonas</i> spp., <i>Ochrobactrum intermedium</i> and <i>Paenibacillus lentimorbus</i>	Offer biotic stress tolerance (biocontrol)	Rice, Wheat, Tomato and Pine	[25-29]

Conclusion

In the current scenario, the agriculture industry not only plays a pivotal role in survival but also facilitates meeting the demands of the growing population and economic exports. PGPR are bacteria possess capability of promoting plant growth by colonizing the plant root. They are an important cluster of beneficial, root-colonizing bacteria thriving in the plant rhizosphere. In previous years, PGPR were mainly considered as boosters for enhancing plant growth by nutrient uptake. Nowadays, PGPR are emerging as stress busters to alleviate effects of stressful environmental conditions in agriculture. Along with it, bioremediation is also new and promising approach to eliminate contaminants in the environment and PGPR have been reported to play a critical role in it. However, their application as biofertilizer for increasing agricultural yield is contributing in sustainable agriculture in an environmental friendly manner. Potential PGPR may offer a widespread cost effective adoption by farmers which further deliberate the cost effective and eco-friendly prospects to promote sustainability in agriculture sector.

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Arbuscular Mycorrhizal Fungi: Role in Sustainable Agriculture

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Introduction

Sustainability in agricultural production has emerged as one of the most significant concerns in the 21st century (Kuila and Ghosh, 2022). Sustainability refers to “successful management of resources for agriculture to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving natural resources” (TAC, 1989). The current day emphasis is on sustainable agriculture, which uses less of chemical inputs having adverse effect on soil health, and environment. The arbuscular mycorrhizal fungi (AMF) and other microbial inoculants play an important role in sustainable agriculture. The term “mycorrhiza” was coined by A. B. Frank (1885) literally means “fungus root.” It is a symbiotic association between plant roots and fungi. Though there are different kinds of mycorrhiza, the most common mycorrhizal association occurring in crops important in agriculture and horticulture is the arbuscular type. These fungi in soil are ubiquitous throughout the world and form symbiotic relationships with the roots of most terrestrial plants (Bagyaraj et al., 2022a).

AMF are said to establish a mutualistic relationship with 80% of vascular plants (Samuel et al., 2021). Plants that rarely form AM fungal association include Caryophyllaceae, Brassicaceae, Chenopodiaceae and Cyperaceae. In addition to their widespread distribution throughout the plant kingdom, arbuscular mycorrhizae are ubiquitous and occur in plants grown in arctic, temperate and tropical regions. They have been reported to be associated with plants growing in sand dunes, coal mines and aquatic environments (Vieira et al., 2019). AMF have the widest

host range and distribution of all the mycorrhizal associations. AMF have been observed in 1000 genera of plants representing some 200 families. There are at least 300,000 receptive hosts in the world flora and there are about 343 species of AMF. If the hosts are divided up evenly among the fungi, with no overlap in host range, each fungus would have more than 875 potential partners. We know that the host range overlaps extensively, suggesting that some individual AMF may well have access to thousands of host. In tropics the most common type of mycorrhizal association is AM type.

The fungi

AMF belong to the phylum Glomeromycota, which has a single class Glomeromycetes with four orders Glomerales, Diversisporales, Paraglomerales and Archaeosporales. There are 12 families, 44 genera and 343 species (http://www.amf-phylogeny.com/amphylo_downloads.html). The commonly occurring genera of AMF are *Glomus*, *Gigaspora*, *Acaulospora*, *Entrophospora* and *Scutellospora*. In the soil, AMF produce large thick-walled resting spores called extramatricular chlamydospores, which can survive adverse soil conditions and germinate when conditions are favorable. The germ tube emerging from spores penetrate root hairs or epidermal cells and then grows intercellularly and / or intracellularly in the root cortex, ultimately developing short haustoria like structures called arbuscules within the cortical cells. These arbuscules function as sites of nutrient exchange between the fungus and host roots. Vesicles are formed in the cortical cells, which are thin walled structures of various sizes and shapes and function as storage organs. The presence of

vesicles and arbuscules is the criteria for identifying AM fungus in the roots. These fungi are obligate symbionts and have not been cultured on nutrient media (Giovannini et al., 2020).

Isolation and maintenance of AMF

A root system colonized by AMF does not show any morphological variations from the normal root system and hence cannot be distinguished visually. In some plant species like onions, maize, clover etc. they may appear yellowish. This colour, however, disappears rapidly when exposed. Hence, mycorrhizal status of a root system can only be known through microscopic observation after staining the roots with trypan blue. AMF can be isolated from soil by the wet sieving and decantation method (Bagyaraj and Sturmer, 2008; Trejo-Aguilar and Banuelos, 2020). Single spores thus obtained can be surface sterilized by immersing in a solution containing 2% (w/v) chloramine T and 200-ppm streptomycin sulphate for 15 minutes, followed by washing 3 times in sterile water. Individual spores can be picked by a fine capillary pipette, under a dissecting microscope and placed in the collar region of a funnel filled with sterile sand and seeded with any suitable host like sorghum or any other graminaceous host. After 3-4 weeks, the seedlings along with sand from the funnel can be transferred to sand: soil (1:1) mixture in a pot and maintained in the glasshouse as 'pot culture'. Once a pot culture of a single species is achieved, it can be used as inoculum to multiply the fungus for future experiments (Nicolson, 1967; Agnihotri et al., 2022).

Plant growth response to inoculation and mechanisms involved

Earlier experiments conducted in sterilized soil showed that AMF inoculation could improve plant growth. Since most of the natural soils usually harbor AMF, it was felt that plants may not respond to mycorrhizal inoculation in unsterile soils. But later investigations indicated that even in unsterile soils, plants do respond to inoculation with

efficient strains of AMF. It is now proved beyond doubt that AMF greatly enhance plant growth. The improved growth is mainly attributed to uptake of diffusion-limited nutrients such as P, Zn, Cu, etc. from soil. The other beneficial effects are their role in the biological control of root pathogens, hormone production, greater ability to withstand abiotic stress, and synergistic interaction with nitrogen fixers, P solubilizers, and plant growth-promoting rhizo-microorganisms (PGPR) (Lehmann et al., 2017; Kuila and Ghosh, 2022).

The role played by these fungi in improving plant growth is much more significant in tropical soils compared to temperate soils. This is mainly because most of the soils of the tropics are of low inherent fertility (Berruti et al., 2016; Bagyaraj et al., 2022a). They are deficient in phosphorus. In addition to being deficient in phosphorus, this nutrient also gets fixed in the soil and is not readily available over the crop period necessitating fresh additions. In acidic soils, they are fixed as iron and aluminium phosphates, while in neutral soils they are fixed as calcium phosphates. Continuous application of P fertilizers will result in increased concentration of total phosphorus in the soil over times, resulting in large reserves of fixed P. According to Ozanne (1980), less than 10% of soil P enters the plant-animal cycle. Experiments with ³²P-labeled phosphorus conclusively proved that AMF cannot solubilize unavailable inorganic phosphorus sources but draw extra phosphate only from the labile pool in soil solution (Raj et al., 1981). The improved P nutrition in plants has been explained mainly by the extension of AMF hyphae beyond the root system which allows for the exploration of spatially unavailable nutrients (Smith et al., 2000; Bagyaraj et al., 2022a). The rate in which plant roots absorb phosphorus from the soil solution is much faster than the rate in which phosphorus moves in soil solution by diffusion. This results in a phosphorus depletion zone around the root. It is here that AMF play the most significant role. The

external hyphae of AMF travel much beyond the P depletion zone, scavenge a large volume of soil and supply P to the plants. In exchange, the AMF receive carbohydrates from its host plant (Bagyaraj and Ashwin, 2017). It can be generalized that plant growth increase is favored in soils with low to moderate fertility, especially phosphorus in limiting concentrations (Dodd and Jeffries, 1986; Etesami et al., 2021).

Anatomical and other physiological studies have brought out that mycorrhizal plants have increased rates of respiration, photosynthesis and increased amounts of sugars, amino acids, RNA etc. and larger and/or more number of chloroplasts, mitochondria, xylem vessels, motor cells, etc. This suggests that mycorrhizal plants are much healthier with better metabolic activity (Anand et al., 2022). Changes in the root exudations and altered rhizosphere microorganisms (which also affect plant growth) may result because of colonization of roots by AMF. Mycorrhizal colonization may also allow introduced populations of beneficial soil organisms like *Azotobacter*, *Azospirillum* and phosphate solubilizing bacteria to be maintained in high numbers than around non- mycorrhizal plants and to exert synergistic effects on plant growth. It is apparent from the investigations on AMF - plant pathogen interaction that AMF can usually deter or reduce the severity of disease caused by soil-borne pathogens. AMF are also known to protect plants against abiotic stresses like drought, salinity, heavy metal toxicity etc. (Zarik et al., 2016; Anand et al., 2022). All these studies bring out that AMF help the host plant in more than one way and that AM fungal inoculation helps plants growth.

Mycorrhizal dependency and selection of efficient AMF for inoculation

Plants differ greatly in their mycorrhizal dependence. Relative mycorrhizal dependency is defined as the degree to which a plant is dependent on mycorrhizal condition to produce maximum growth or

yield at a given level of soil fertility (Berch et al., 1985; Bagyaraj et al., 2022a). Based on this, plants can be categorized as highly dependent, moderately dependent, less dependent or not dependent on mycorrhizal fungi. It is always advantageous to categorize plants in a region according to their mycorrhizal dependency and work on those which are highly dependent in order to get better results (Plenchette et al., 1983). It is well known that AMF are not host specific. Though a particular AM fungus can infect and colonize many host plants, it has a preferred host, which exhibits maximum symbiotic response when colonized by that particular AM fungus (Abbott and Robson, 1982; Bagyaraj, 2014a). This led to the concept of 'host preference' in AMF and in turns the procedure for screening and selecting an efficient fungus for a particular host. This in turn led to the selection of inoculant AMF for many crops important in agriculture, horticulture and forestry (Bagyaraj and Kehri, 2012; Parab et al., 2013; Anand et al., 2022).

Use of AMF for plant growth

AMF inoculum of suitably selected strains can be used for inoculation in the nursery bed (Palazzo et al., 1994). Growers only need to incorporate inoculum in the nursery beds or seedling trays at the appropriate rate by hand. Seedlings thus raised will be colonized by the introduced fungus and then can be planted out in the field. For directly field sown crops AMF inoculum can be mixed with compost and applied to the furrows before sowing or transplanting the crops. There are several reports of increased growth and yield of food, fodder, and fuel crops because of inoculation with efficient AMF (Berruti et al., 2016; Bagyaraj et al., 2022a). These studies also brought out that because of inoculation, nearly 50% of phosphate fertilizer application could also be reduced (Thilagaret al., 2016; Anuroopaet al., 2017; Jyothi et al., 2018). Some horticultural plants are propagated through cuttings. In such cases, rooting of cuttings is important. Enhanced rooting of cuttings

through inoculation with AMF has been reported. AMF-inoculated plants withstanding transplant stock have also been reported in avocado, **sesbania** and cashew (Mengeet al., 1980; **Subhan et al., 1998; Lakshmipathy et al., 2000**). Later studies showed high percentage of grafting success in cashew (Lakshmipathy et al., 2000). Inoculation of micropropagated plantlets with AMF after hardening also improved plantlet vigor and growth in coffee, grapevine, apple, avocado, pineapple, kiwi fruit, strawberry, raspberry, asparagus, and banana (Yao et al., 2002; Bagyaraj, 2014b). The information available as to whether perennial plants already established in the field respond to AMF inoculation is very meager. In a study, it was found that ten-year-old mulberry plants and one-and-half-year-old papaya trees positively responded to mycorrhizal inoculation (Mamatha et al., 2002). Thus, use of AMF can be considered as an alternate strategy to more rational and sustainable agriculture.

Application methods and time of application

It is important to standardize the dose and time of application, apart from the method of application, in order to harness the maximum benefit from the AM-host association (Thirkell et al., 2017). In a potential inoculum, every propagule is capable of colonizing the host root. However, in order to ensure a threshold level of colonization and quicken the process of colonization, a higher density of propagules should be present in the inoculum. However, the number varies with other factors, such as their ability to tolerate soil (pH, nutrient status) and environmental factors (light intensity, temperature) and to proliferate. In horticultural/forest tree species, where planting is carried out at comparatively larger distances, high inoculum density is preferred, unlike in other agricultural crops where seedling density is high, hence roots have a greater probability of encountering the infective propagules. For raised nursery beds 500g of inoculum per one sq meter can be applied 2-3 cm below

the soil. For plants raised in polybags about 5g of the inoculum can be applied 6 cm below the soil. For directly field sown crops 4-5kg of the inoculum can be mixed with compost and applied in the furrows.

Considering the time of application, it has been well demonstrated that earlier the inoculation, the greater the benefits to the host. Earlier studies reveal that inoculation at the time of sowing/planting will result in better colonization of the roots as they emerge, hence help in better establishment of the host. Experiments conducted with micropropagated *Ficus benzamina* have revealed that the best time for inoculation would be just before planting out hardened plantlets (Shashikala et al., 1999). Methods of application of AMF generally include hand placement placing the inoculum few cm below the seed or seedling. For directly filed sown crops AMF inoculum can be mixed with compost and applied to the furrows before sowing or transplanting the crops. The importance of method of application of inoculum arises with the need to initiate colonization in the early stages of plant growth. Hence, from this view point, it is essential to place the inoculum close to the seed material such that the roots will come into contact with the fungal material as soon as they emerge. Based on an experiment it was concluded that placing AM inoculum 8cm below the surface of the soil (7cm below the seed), at a point or as a layer, was the best method of inoculum placement for seedlings raised in polybags. For seedlings raised on the nursery beds, placing the inoculum 2 cm below the seed was found to be the best approach (Bagyaraj, 2015).

Inoculation of AMF along with other beneficial soil microorganisms

There are several reports on the interaction between AMF and other beneficial soil microorganisms like nitrogen fixers, phosphate solubilizers and other PGPR. These studies suggest that the interaction is synergistic with consequential benefit on plant growth. Recent studies have shown that inoculation with microbial consortia

consisting of an efficient AM fungus together with a nitrogen fixer, P solubilizer and other PGPR carefully screened and selected for a particular crop plant or forestry species is more beneficial than AM fungus alone in improving the growth, biomass and yield. Recently such microbial consortia for inoculating crop plants and tree species have been developed after careful screening under polyhouse conditions followed by large scale nursery trials (**Raklami et al., 2019; Laranjeira et al., 2022**). The fundamental means of assessing the effectiveness of inoculating the nursery seedlings are their survival and growth in the field.

In the case of leguminous plant seedlings, joint inoculation of symbiotic nitrogen fixing bacteria rhizobia and AMF proved to be very useful. There are several reports on the interaction between AMF and rhizobia. These studies suggest that the interaction is synergistic; improving nodulation and AM fungal colonization, with consequential benefit to plant growth (**Igiehon et al., 2021**). Similar results have also been reported on other associative nitrogen fixing bacteria like *Azospirillum* and free-living nitrogen fixing bacteria like *Azotobacter* (**Amirnia et al., 2019; Bagyaraj, 2018**). It is well known that N-fixation has a high P requirement and that is provided by AMF. Interaction between AMF and P solubilizing microorganisms (PSM) have been studied by several workers. The relationship was found to be synergistic and dual inoculation helped plant growth better compared to single inoculation with either of them. PSM helped in solubilizing unavailable form of P in soil to an available form and AMF helped in the uptake of available P for plant growth. Recent studies have shown that inoculation with microbial consortia consisting of an efficient AM fungus together with a nitrogen fixer, P solubilizer and PGPR carefully screened and selected for a particular crop plant or forestry species is more beneficial than AM fungus alone in improving the growth, biomass and yield. Results of field experiments conducted with vegetable crops chilli and French bean,

and medicinal plants ashwagandha, tulasi support this. Further these studies also brought out that 50% of NPK fertilizer can be saved through inoculation with the selected microbial consortia with no adverse effect on crop yield (**Wang et al., 2020; Biel et al., 2021**). In a recent study forest tree seedlings (*Acacia auriculiformis* and *Tectonagrandis*) inoculated in the nursery with microbial consortia consisting of selected AMF and PGPR were planted in degraded forests. Inoculated plants survived and grew much better compared to uninoculated plants 54 months after planting. The size of inoculated plants was almost double the size of uninoculated plants (Raghu et al., 2020).

Role of AMF in the biocontrol of soil-borne plant pathogens

There are several reports on AMF-soil-borne plant pathogen interactions (Bagyaraj, 2016; Singh et al., 2019). Role of AMF in improving plant growth is now well documented. Most of the studies on AMF-root pathogens suggest that AMF decreased or mitigated the disease severity. Consistent reduction of disease symptoms has been described for fungal, bacterial and nematode pathogens. Studies conducted so far suggest that the mechanisms of suppression may be due to morphological, physiological and biological alterations in the host (Frac et al., 2018). Thickening of the cell walls through lignification and production of other polysaccharides in mycorrhizal plants preventing penetration and growth of pathogens like *Fusarium oxysporum* and *Phomaterrestris* have been demonstrated. Higher concentration of ortho-dihydroxy phenols present in mycorrhizal plants compared to non-mycorrhizal plants was found to be inhibitory to the root rot pathogen *Sclerotium rolfsii*. The activation of specific plant defence mechanisms as a response of AMF colonization is an obvious basis for the protective capacity of AMF. Among the compounds involved in plant defense studied in relationship to AMF formation are phytoalexins, enzymes of the phenylpropanoid pathway, chitinases,

peroxidases, pathogenesis related (PR) proteins etc. Mycorrhizal plants harbour higher population of microorganisms in the rhizosphere thus making it difficult for the pathogen to compete and gain access to the root. Further mycorrhizosphere supports higher population of antagonists and siderophore producers. Thus, the possibility of biologically controlling the root pathogens with AMF looks promising (**Aljawasim et al., 2020**).

Mass production of AMF

Attempts to culture AMF on artificial media have met with little or no success. At the present time, the only method to produce these fungi is in association with the host plant roots. Novel techniques to produce AM inoculum in almost sterile environment through nutrient film technique or circulatory hydroponic culture system have not reached commercialization because of many limitations. Large-scale production of AM inoculum is technically feasible using traditional pot culture technique developed few decades back by Barbara Mosse and Jim Gerdemann (Khaliq et al., 2010; **Selvakumar et al., 2016**).

a) Substrate based inoculum

Substrate based inoculum produced using traditional "Pot Culture" technique contains all AM fungal structures and is highly infective (Trejo-Aguilar and Banuelos, 2020). The success of good substrate-based inoculum production depends on selection of host plant and ambient conditions under which a defined AMF can be mass multiplied. A series of experiments were conducted and it was found that Rhodes grass is the best host, vermiculite : perlite : soilrite as the best substrate and calcium ammonium nitrate and rock phosphate to be the best N and P sources for mass producing *Glomus fasciculatum* (Sreenivasa and Bagyaraj, 1990). Further, modified Ruakura nutrient solution added once in 8 days and harvesting pot cultures in 75 days produced high quality inoculum with maximum number of infective propagules. Addition of captan and

carbofuran to the substrate at half the recommended level significantly reduced the contaminant fungal and nematode population. The inoculum thus produced contained nearly 2000 infective propagules per gram of the substrate with least contaminants (Sreenivasa and Bagyaraj, 1990). Later studies have shown that soilrite TC (tissue culture) grade made up of perlite + *Sphagnum* moss (1:3) is better than soilrite SP (special) grade which is made up of perlite + vermiculite + *Sphagnum* moss (1:1:1). **Substrate based inoculum is a cost-effective method to mass produce AMF spores for large-scale application (Selvakumar et al., 2016)**. Further, addition of plant growth promoting rhizomicroorganisms was found to increase the number of infective propagules of AMF. The shelf life of substrate-based inoculum was found to be nearly 2 years at room temperature, hence there is no need to store the inoculum under refrigerated condition (Harinikumar et al., 1992).

b) Root organ culture

Agrobacterium rhizogenes is a soil-borne pathogen causing "hairy root" in dicotyledonous plant. When wounded tissues are infected with the bacteria, they form large number of hairy roots. Stem explants of chicory and tomato were found to be the best suited for root induction. The transformed roots can be sub-cultured as excised root. The desirable characteristic of this transformed root is the ability to quickly form numerous lateral roots. They also survive for a longer period without subculture. Such root organ cultures have been inoculated with surface sterilized AM spores. Studies have been conducted to continuously maintain AMF in root organ culture. This is the best method for maintaining AMF as pure cultures that can be used for genetic studies. The disadvantages are lesser shelf life and only limited AMF can be mass produced by this method (Khaliq et al., 2010; **Agnihotri et al., 2022**).

Quality Assessment of AMF Inoculum

In the recent past, the mass production of arbuscular mycorrhizal (AM) fungi has bloomed into a large biofertilizer industry. Due to their obligate symbiotic nature, these fungi are mostly propagated on living roots in substrate-based pot cultures and to a limited extent through Ri T-DNA in *in vitro* or root organ culture systems. The quality assessment of AM inocula remains critical for the production and efficacy evaluation of AMF. The determination of AM fungal inoculum quality is a critical aspect of inoculum production. It should be a prerequisite to test the inoculum potential of AMF inoculum under microcosm and field trials through consistent screening protocols and confirm a sizeable AM fungal propagule density, before making them commercially available.

The quantification of AMF requires considerable expertise. Conventional microscopic quality assessment methods viz. spore numbers and infective propagule numbers/g are commonly used for assessing the quality of the inoculum. Few biochemical (phospholipid fatty acid profiling) and molecular methods (qPCR) have been attempted as newer methods and are still in research stage. Lack of primers, signature fatty acids representing more than one microbial species and presence of different infectious propagules by species within root segments is the most frequently encountered problems in the newer methods (Agnihotri et al., 2022). *In vitro* production of AMF ensures the purity of isolates but remains technically rigorous and as of today all AM fungal species cannot be produced by this method. Substrate-based production stands as a method of choice for mass-producing AMF in developing countries, primarily as these inocula remove the handling and shipping prices and maintain viability at room temperatures (Douds et al., 2005). Considering the pros and cons of the different methods available for testing the quality of commercially produced AMF inoculum, determining the IP numbers/g by

MPN method with 10-fold dilution appears to best reflect the quality of the inoculum (Agnihotri et al., 2022). In India, according to the fertilizer control order (FCO) specifications, AM fungal biofertilizers should contain 10 viable spores and 1200 infective propagules (IP) per gram inoculum (The Gazette of India no. 2473, The Fertilizers Fifth Amendment Order, July 2021).

Conclusion

Chemical fertilization has been used in agriculture over the past few decades to increase the crop yield, which created issues like environmental pollution and degradation of soil health. Hence, at present considerable importance is given to sustainable agriculture which uses less chemicals and more biologicals like compost, biofertilizers, biopesticides etc. AMF is one of the biofertilizers used. The role of AMF in improving plant growth is well documented. The beneficial effects includes improvement in the uptake of diffusion-limited nutrients, synergistic interactions with beneficial soil microorganisms, production of plant growth promoting substances, and greater ability to withstand water, salinity, heavy metal stress and root pathogens. One of the major constraints in the utilization of AMF is the poor quality of the commercial inoculum. Most of the farmers in the country do not have sufficient and clear knowledge on the use of AMF. The farmers need to be educated. This can be done through demonstration trials on farmers field and publicity programmes through media like TV, write-ups in local papers, seminars etc. Use of AMF as biofertilizers will not only improve plant growth and productivity but will also reduce the use of fertilizers and pesticides thus minimizing environmental pollution.

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Method and application of Blue Green Algae in agricultural field

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Introduction

Blue green algae (BGA), also known as cyanobacteria, blue-green bacteria, and Cyanophyta, is heterogeneous group of Gram-negative prokaryotes, perform oxygenic photosynthesis by capturing sunlight for energy using chlorophyll *a* and various accessory pigments, and also fix atmospheric N₂, the most successful and oldest life forms present on the planet earth. They comprise of about 150 genera and 2,000 species, some of the predominant nitrogen fixing genera are *Anabaena*, *Nostoc*, *Aulosira*, *Calothrix*, *Tolypothrix*, *Aphanothece* and *Gloeotrichia*.

2. Characteristic Features of Cyanobacteria/Blue Green Algae

2.1 Cyanobacterial- Plant Associations

Many cyanobacteria form symbioses with selected prokaryotic and eukaryotic organisms including all kingdoms viz., bacteria, lichenized and non-lichenized fungi, photosynthetic algae, lower and higher plants, and a few smaller animals such as sponges, ascidians (either sea squirts or tunicates) and some worms. Cyanobacteria with symbiotic competence are primarily found in the genus *Nostoc*, which is common in **and** terrestrial environments, although a few other heterocystous cyanobionts (symbiotic cyanobacteria), such as *Calothrix* *Scytonema* (in lichens and cycads) and *Richelia* (in diatoms), also form symbioses. Non-heterocystous cyanobacteria, such as *Oscillatoria*, *Phormidium*, and unicellular cyanobacteria of the genus *Prochloron*, *Aphanocapsa*, *Synechocystis* are also reported as capable of associating with marine eukaryotes. All hosts that harbor heterocystous cyanobacteria (e.g., *Nostoc*,

Calothrix, *Scytonema*, and *Richelia*) rely on the N₂-fixation capacity of the cyanobacteria to cover their need for combined nitrogen.

3. Nitrogen Fixation in Cyanobacteria

In addition to being photosynthetic, many species of cyanobacteria can also “fix” atmospheric nitrogen *i.e.*, they can transform the gaseous nitrogen of the air into compounds that can be used by living cells. They have specialized nitrogen-fixing cells called heterocysts.

3.1 Heterocysts Structures and Features

Heterocysts are specialized cells acts as site for nitrogen fixation in filamentous multicellular cyanobacteria. It is distinguished from vegetative cells by their thick wall, polar nodule(s) and homogenous contents. Heterocysts are usually found in the members of *Stigonematales* and *Nostocales* (except *Oscillatoriaceae*). They may be either terminal (*Gloeotrichia* and *Calothrix*) in position or intercalary (*Nostoc*) or the genus may have both terminal and intercalary (*Anabaena desikacharyiensis*). It is pale yellow, thick-walled specialized cells, larger than vegetative cells and surrounded by “complex multilayered envelope” which is differentiated into three regions, an outer fibrous, middle homogenous and inner lamellar layer.

4. Role of Cyanobacteria in Agriculture and Environment

Cyanobacteria are the effective tool for enhancing the soil fertility, bio-fuel production, bioremediation, reducing GHGs emissions and enhancing crop productivity. The application of cyanobacteria in management of soil and environment includes the economic benefits, organic

wastes decomposition, heavy metals detoxification, bioavailability of nutrients, environmental protection and prevention of pollution and land degradation especially through reducing the use of agro-chemicals, and recycling of nutrients and restoration of soil fertility through reclamation. The following are the benefits of cyanobacteria to agro-ecosystem:

- Acts as source of natural pigments, nutritional supplements, pharmaceuticals/drugs, Biofuel etc.
- Good food source for aquatic animals; exploited as food for animals including humans eg. *Spirulina*.
- They are also N₂-fixers and save consumption of N-fertilizers for crop production.
- They can be used for reclamation of user soil.
- Also used as mosquito larvicides eg. *Aulosira* and *Anabaena*.
- Provide protection to plants from pathogenic insects and diseases as bio-control agents eg. *Calothrix* and *Lyngbya*.
- Enhanced solubilization and mobility of nutrients and make easily available to plants.
- Complexing of heavy metals and xenobiotics to limit their mobility and transport in plants.
- Also produces antibacterial or antifungal substances that and provide protection to plants from pathogens.
- Excretion of growth-promoting substances such as hormones (auxin and gibberellin), vitamin B12, amino acids like alanine, aspartic acid, glutamic acid, and sugars which can be beneficial for crop plants.
- Excretes polysaccharides and improve soil aggregation and reduces soil

compaction, increases pore size, aeration and water holding capacity.

- Can solubilize the insoluble phosphates in the soil by excretion of organic acids.

5. Isolation of cyanobacteria from soil and water samples

5.1 Culture media

Generally, BG-11 medium without N is used for isolation of nitrogen fixing and with N for non-nitrogen fixing cyanobacteria.

5.2 Sterilization of the medium and glassware

The medium is sterilized at 15 lb/in² pressure for 20 minutes at 121 °C in an autoclave.

5.3 Enrichment cultures

The soil samples weighed in 5 g portions are transferred to 100 ml sterilized medium in 250 ml conical flasks. The flasks are shaken well and incubated undisturbed in a growth room under optimal growth conditions for 20-25 days at 28±2°C temperature and 4-5 K lux light intensity.

5.4 Isolation

The incubated flasks are observed daily for the algal growth and when visible algal growth appears 6-7 wet mounts from each flask are prepared lifting the algal growth from the surface of soil, water and walls of the container for microscopic examination. It is suspended in 5 ml sterilized distilled water in test tubes, shaken vigorously to make a homogenous suspension and streaked on agar plate medium accordingly to the observations made *i.e.* +N (with N₂ source) agar plate medium for non heterocystous forms and -N (without N₂ source) agar plate medium for heterocystous forms. The plate is observed regularly and isolated colonies are picked up and examined under microscope. If found unialgal another similar colony is picked up from the plate and transferred to agar slant. Further biochemical characterization of axenic cultures can be done.

6. Cyanobacteria used as Bio-fertilizer in rice cultivation

Efficient nitrogen fixing strain like *Nostoc linkia*, *Anabaena variabilis*, *Aulosira fertilissima*, *Calothrix* sp., *Tolypothrix* sp., and *Scytonema* sp. were identified from various agro-ecological regions and utilized for rice production. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural bio-fertilizer. They have potentiality to fix large amount of atmospheric nitrogen (up to 20 - 25 kg/ha). Genera *Nostoc*, *Anabaena*, *Tolypothrix* and *Aulosira* are used as inoculants for paddy crop grown both under upland and low land conditions. Cyanobacteria play following important role in maintenance and buildup of soil fertility, consequently increasing rice growth and yield as a natural bio-fertilizer:

- Diazotrophic cyanobacteria maintain and increase the soil fertility of rice fields.
- Reduce the nitrogenous consumption by 15-30 percent (20-30 kg N/h/season).
- Increase productivity of rice by 10-15%.
- Algalization induces early grain setting and maturity.
- Checks weeds proliferation by blocking nutrient supply and light.
- Increase the utilization of N-fertilizer by partially reducing the losses through run-off, leaching and denitrification.
- Buffers the soil against rapid changes in pH.
- Reported to reduce the salinity in the soil leading to better crop response.
- Enhance the activity of other beneficial micro-flora and increases population.
- Protection of plants from pathogenic insects and diseases as bio-control agents.
- Mineralization of simpler organic molecules such as amino acids for direct uptake.

- Increase in soil pores with having filamentous structure and production of adhesive substances.
- Increase in soil biomass after their death and decomposition.

7. Different methods for BGA mass production

Presently, several technologies are available, such as indoor technology and production is in defined growth media. Most commonly used species in bio fertilizer production are *Anabaena variables*, *Nostoc muscorum*, *Aulosira fertilissima*, and *Tolypothrix tenuis*.

7.1 Mass cultivation of cyanobacteria for bio-fertilizer production

The pure culture of BGA are transferred to culture flask having BG-11 (-N) medium for growth and used as a starter culture to initiate the mass culture of BGA. The algal production technology developed and reported by different algologists is very simple in operation and easy in adaptability by Indian farmers. The technology has got potential to provide an additional income from the sale of algal bio-fertilizer. In general, there are four methods of algal production have been reported viz.,

- (1) Trough or tank method,
- (2) Pit method,
- (3) Field method and
- (4) Nursery cum algal production method.

The former two methods are essentially for individual farmers and latter two are for bulk production on a commercial scale.

8. Cyanobacterial Bio-fertilizer Production Technology

After harvesting, the harvested fresh biomass weighed and mixed with equal amount of Multani mitti (1:1 ratio) or straw and well dried in sun as thin layer. Well dried mixture then grinded (Mesh size: 200) and packed suitably in polythene bags @ 500 g/pack and quality assessment. The bags are stored in cool dry place. These BGA can

be preserved for 3 years without losing its efficiency.

9. Methods of Application of BGA Bio-fertilizer

One packet (500 g) of ready to use Multani mitti based BGA bio-fertilizer is recommended for one acre of rice growing area. Bio-inoculant is mixed with 4 kg dried and sieved farm soil and broadcast on standing water at 3-6 days after transplantation. The field should be kept waterlogged for about 10-12 days after inoculation to allow good growth of BGA. When nitrogenous fertilizers are used, reduce the dose by one-third and supplement with BGA. Apply BGA for at least four consecutive seasons to have cumulative effect.

Precautions: When fertilizer or pesticides (e.g. weedicides) are applied in the field; the algal application should be followed after a gap of 3-4 days. Application of a small dose of phosphate fertilizer after BGA inoculation accelerates BGA multiplication.

10. Advantages of Cyanobacterial Bio-fertilizers

Bio-fertilizers are becoming a rage, considering the irreparable damage that the chemical fertilizers are causing to the soil. Some of the advantages associated with bio-fertilizers include:

- The first and the most important advantage of using bio-fertilizers is that they are environment friendly, unlike chemical fertilizers that damage the environment.
- They are comparatively low on cost inputs and are light on the pockets of the farmers.
- Bio-fertilizers improve root proliferation due to the release of growth promoting hormones
- They help in increasing the crop yield by 10-25%.

- They are having higher photosynthetic efficiency, faster growth and easily mass cultured.
- Biomass conversion efficiency is 18% due to the microscopic size which increases the surface area.
- Can be grown round the year; require less area, simple and easily available cheap nutrients.
- Entire biomass can be used without any processing.
- Non-polluting, environmentally friendly and ecologically safe for nature.

11. Limitations of Cyanobacteria/BGA Bio-fertilizer

- Lack of regulatory acts and facilities for testing the samples.
- Insufficient popularization of bio-fertilizers and low level of farmer acceptance.
- Decline in the population of bacteria under certain climate conditions and influence of surrounding microflora and fauna.
- Application in fields needs manpower.
- Bio-fertilizers are not readily accepted by the society primarily because they do not produce quick and impressive responses.
- Require special care for their long term storage.
- Soil must contain sufficient nutrients to facilitate the working and effectiveness of bio-fertilizers.
- Bio-fertilizers lose their quality when the soil is dry or hot or adverse condition.
- Bio-fertilizers will become ineffective when the soil contains natural microbes.

12. Future Prospects of Cyanobacteria/BGA bio-fertilizers

- More focus should be on selection of region specific effective species of

cyanobacteria and bio-fertilizer inoculants should be produced according to climate situations.

- Quality control system is much needed for quality certification, production and their application in the field.
- Agronomic and economic evaluation of cyanobacterial strains for diverse crops is required.
- Establishment of “Bio-fertilizer Act” and strict regulation for quality control of bio-fertilizers is required to make BGA and other fertilizers acceptable to farmers.
- A single commercially viable method especially liquid based formulation of BGA is much needed to develop.
- Extensive field trials for awareness building should be done.

Conclusions

BGA bio-fertilizers have various benefits. Besides accessing nutrients, for current intake as well as residual, they also provide growth-promoting factors to plants and facilitate composting and effective recycling of solid wastes. They also provide protections from diseases, improving the soil health and help not only in saving, but also in effectively utilizing chemical fertilizers and result in higher crop yield. Cyanobacteria play a spectrum of remarkable roles in the

field of bio-fertilizer, energy production, human food, animal feed, polysaccharides, biochemical, pharmaceutical and changing up of the environment, etc.

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National and International News and Events on Organic Farming

1) Participation in Webinar on “Indian Standards on Biofertilizers” Organized by BIS

Dr. Gagnesh Sharma, Director (I/c), NCONF Ghaziabad attended the webinar held on 24.01.2022 and delivered expert talk on Biofertilizer standards as per FCO 1985. He also addressed many issues raised by the stakeholders or participants during the webinar.



2) Republic Day Celebrations at N/RCONFs Premises

Officials of N/RCONFs celebrated Republic Day with great patriotic Zeal and fervor in Centres' located at Ghaziabad, Bengaluru, Bhubaneswar Nagpur, Gandhinagar, Jabalpur Panchkula, Patna and Imphal sprawled across the country.



3) Agriculture Workshop at IIT Roorkee

Dr. S.K. Vaid, JSO, NCONF Ghaziabad and Dr. A. Singla, JSO of RCONF Ghaziabad organized agriculture workshop on organic farming under

Samarthya at IIT Roorkee on April 02, 2022. NCONF Officials delivered expert talks on various aspects of organic farming, its management, certification and marketing etc. The workshop was attended by farmers, members of FPOs and by various other stakeholders.



4) Farmers' Visit at NCONF Ghaziabad

Farmers under Punjab State Rural Livelihood Mission visited NCONF Ghaziabad on Feb. 28, 2022. A day long visit includes series of lectures and field visits by officials of NCONF Ghaziabad including deliberation of keynote address by Director, NCONF.



5) NCONFIA-2022

C.S. Azad University of Agriculture & Technology, Kanpur has organized a two days long “National Conference on Organic and Natural Farming in Context to Indian Agriculture” from April 16-17, 2022 covering a series of topics starting from Human, Soil and Environment Health

Issues to Post Harvest Management and value addition.



6) **BioAg ASIA 2022-** Agriculture Today Group has organized a two days long BioAg ASIA 2022 conference from April 20-21 at NAAS Complex, IARI, New Delhi. The conference aimed to provide a global platform to industry, institutions, experts, officials and international bodies to showcase their potential, achievements, opportunities and scope to connect farmers with agri-startups. Director (I/c), NCONF Ghaziabad was one of the panelist to address the issues and concerns raised by various stakeholders associated with the organic and natural farming.

7) Global Organic Expo-2022

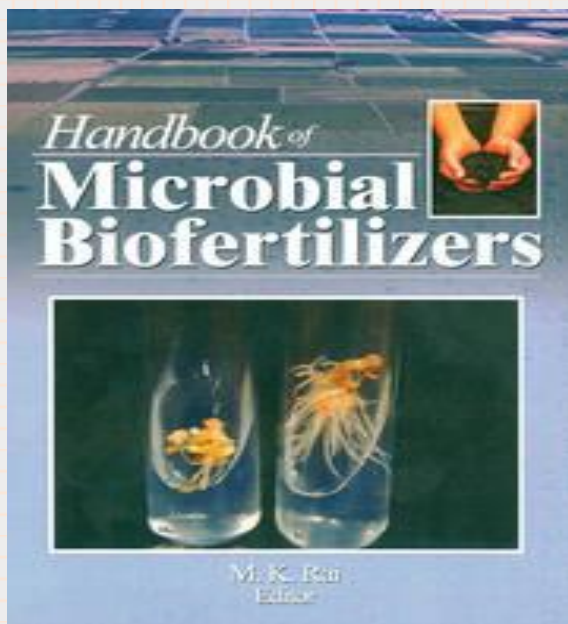
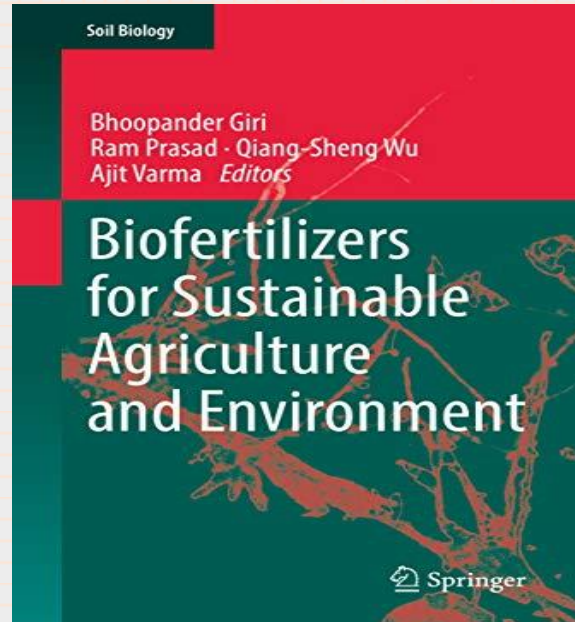
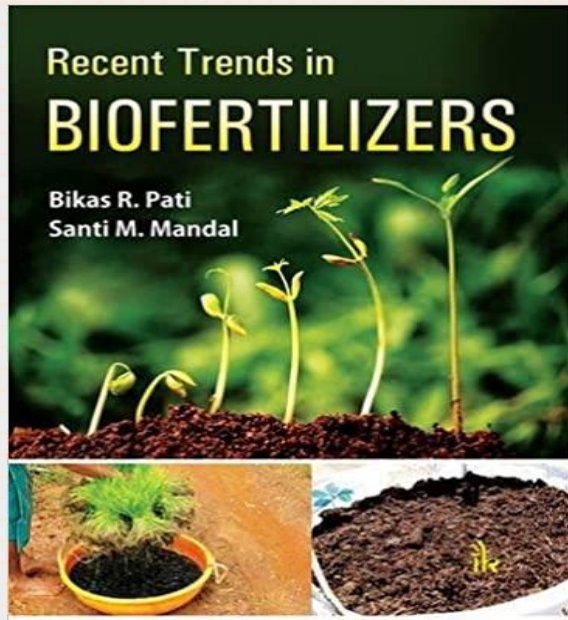
The 3rd Global Organic Expo was organized by Society of Health, Environment, Safety and Sustainability Professionals in IARI, New Delhi from 26 to 28 May 2022. Expo focused on variety of themes of organic agriculture like farming, innovations, Technology, Skill Development, marketing, Distribution and exports.



List of upcoming International Events:

AUG	7 th Asian PGPR International Conference for Sustainable Agriculture https://www.pgpr.org.my/
23 rd - 26 th	
AUG	International Conference on Biofertilizer Technology and Quality Control (ICBTQC) - Kuala Lumpur, Malaysia https://waset.org/biofertilizer-technology-and-quality-control-conference-in-august-2022-in-kuala-lumpur
30 th - 31 st	
SEP	7 th National Asian PGPR Conference for Sustainable & Organic Agriculture - Hyderabad, India https://loyolaacademy.edu.in/wp-content/uploads/2022/07/7th-Asian-PGPR-India-Chapter-National-Conference-at-Loyola-Academy-September-5-6-2022-Final.pdf
05 th - 6 th	
SEP	International Conference on Biofertilizer Technologies and Research (ICBTR) - Dubai, United Arab Emirate https://waset.org/biofertilizer-technologies-and-research-conference-in-september-2022-in-dubai
27 th - 28 th	
OCT	International Conference on Biofertilizer Technologies (ICBT) - Lisbon, Portugal https://waset.org/biofertilizer-technologies-conference-in-october-2022-in-lisbon
27 th - 28 th	
NOV	International Conference on Biofertilizer Technologies (ICBT) - Venice, Italy https://waset.org/biofertilizer-technologies-conference-in-november-2022-in-venice
10 th - 11 th	
DEC	International Conference on Biofertilizer Technology and Organic Farming (ICBTOF) - Auckland, New Zealand https://conferenceindex.org/event/international-conference-on-biofertilizer-technology-and-organic-farming-icbtof-2022-december-auckland-nz
02 nd - 3 rd	

Latest Publications on Biofertilizers



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Name _____

<p>राष्ट्रीय एवं क्षेत्रीय जैविक एवं प्राकृतिक खेती केन्द्रों के पते और उनके कार्यक्षेत्र राज्य</p> <p>Address of National and Regional Organic & Natural Farming Centres with states of their jurisdiction</p> <p>टोल फ्री नम्बर Toll Free Number : 1800-180-3049</p>	
<p>निदेशक राष्ट्रीय जैविक खेती केन्द्र सेक्टर 19, हापुड़ रोड, कमला नेहरू नगर, गाजियाबाद-201 002 (उ.प्र.) 0120-2764906, 2764212; Fax:0120-2764901 वेबसाइट : http://ncof.dacnet.nic.in ईमेल : nbdc@nic.in</p>	<p>Director National Centre of Organic Farming, Sector 19, Hapur Road, Kamla Nehru Nagar, GHAZIABAD - 201 002 (UP). 0120-2764906, 2764212; Fax:0120-2764901 Web: http://ncof.dacnet.nic.in Email: nbdc@nic.in</p>
<p>क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र (मुख्यालय) सेक्टर 19, हापुड़ रोड, कमला नेहरू नगर, गाजियाबाद-201 002 (उ.प्र.) 0120-2764212; Fax:0120-2764901 ईमेल : rcofhq.gzb-agri@gov.in</p> <p>उत्तर प्रदेश, उत्तराखंड, हरियाणा, पंजाब, हिमाचल प्रदेश, जम्मू एवं कश्मीर, लदाख, दिल्ली, राजस्थान और चंडीगढ़</p>	<p>Regional Director Regional Centre of Organic Farming (HQ), Sector 19, Hapur Road, Kamla Nehru Nagar, GHAZIABAD - 201 002 (UP). 0120-2764212; Fax:0120-2764901 Email: rcofhq.gzb-agri@gov.in</p> <p>Uttar Pradesh, Uttarakhand, Haryana, Rajasthan, Punjab, Himachal Pradesh, Jammu & Kashmir, Ladakh, Delhi and Chandigarh.</p>
<p>क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र कन्नामन्गला क्रॉस, व्हाइट फील्ड – होसकोटे रोड, काडुगोडी पोस्ट, बेंगलूरु-560 067 (कर्नाटक) 080-28450503 ईमेल : biofkk06@nic.in,</p> <p>कर्नाटक, केरल, तमिलनाडु, गोआ, दमन एवं दीव, पांडिचेरी तथा लक्षद्वीप</p>	<p>Regional Director Regional Centre of Organic Farming, Kannamangala Cross, Whitefield – Hosekote Road, Kadugodi Post, BENGALURU-560067 (Karnataka). 080-28450503 Email: biofkk06@nic.in,</p> <p>Karnataka, Kerala, Tamilnadu, Goa, Daman & Diu, Pondicherry and Lakshdweep</p>
<p>क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र प्लॉट 23-पी, खण्डागिरी-चंदका रोड, कलिंगा स्टूडियो चौक के पास, घटीकिया, भुवनेश्वर-751 003 (उड़ीसा) 0674-22954958 ईमेल : biofor04.or@nic.in</p> <p>उड़ीसा, पश्चिम बंगाल, सिक्किम, बिहार, झारखण्ड एवं अंडमान निकोबार द्वीपसमूह</p>	<p>Regional Director Regional Centre of Organic Farming, Plot No.23(P), Khandagiri-Chandka Road, Near Kalinga Studio Chowk, Ghatikia, BHUBANESHWAR-751003 (Orissa). 0674-2954958, Email: biofor04.or@nic.in</p> <p>Orissa, West Bengal, Sikkim, Bihar, Jharkhand, Andman & Nicobar Islands.</p>
<p>क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र लांगोल रोड, लाम्फलपेट, इंपाल-795 004 (मणिपुर) 0385-2413239 ईमेल : biofmn01@nic.in</p> <p>आसाम, अरुणाचल प्रदेश, मेघालय, मिजोरम, मणिपुर, नागालैंड और त्रिपुरा</p>	<p>Regional Director Regional Centre of Organic Farming, Langol Road, Lamphelpat, IMPHAL-795 004 (Manipur). 0385-2413239 Email: biofmn01@nic.in</p> <p>Assam, Arunachal Pradesh, Meghalaya, Mizoram, Manipur, Nagaland and Tripura.</p>
<p>क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र अमरावती रोड, राष्ट्रीय राजमार्ग 6, ग्राम – गोंडखेरी, पोस्ट – वाडी, कलमेश्वर, नागपुर-440 023 (महाराष्ट्र) 07118-297054 ईमेल : biofmh10@nic.in</p> <p>मध्य प्रदेश, छत्तीसगढ़, गुजरात, महाराष्ट्र, आन्ध्र प्रदेश, तेलंगाना और दादर एवं नगर हवेली</p>	<p>Regional Director Regional Centre of Organic Farming, Amravati Road, NH6, Village – Gondkhairy, Post – Wadi, Kalmeshwer, NAGPUR-440 023 (Maharashtra). 07118-297054, Email: biofmh10@nic.in</p> <p>Madhya Pradesh, Chhattisgarh, Gujrat, Maharashtra, Andhra Pradesh, Telengana and Dadar & Nagar Haveli.</p>
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