

Bio-fertilizers Role as Key Player in Sustainable Agriculture.

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ABSTRACT: *Current soil management methods rely heavily on inorganic chemical-based fertilizers, posing a significant health and environmental risk. Because of their potential significance in food safety and sustainable crop production, the use of beneficial microorganisms as a biofertilizer has become more important in the agricultural industry. Plant growth promoting rhizobacteria (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria, and many other beneficial microscopic organisms were used in a variety of ways to enhance nutrient absorption, plant growth, and plant tolerance to abiotic and biotic stress. The current review focused on the role of biofertilizers in crop functional traits such as plant growth and productivity, nutrient profile, plant defense and protection, with a particular focus on their ability to trigger various growth- and defense-related genes in the signaling network of cellular pathways, resulting in cellular response and, as a result, crop improvement. The information acquired from the literature reviewed here will aid us in better understanding the physiological underpinnings of biofertilizers, which will aid in the development of sustainable agriculture by minimizing the issues connected with chemical fertilizer usage.*

Keywords: *Agriculture, Biofertilizer, Crop Improvement, Environmental, Sustainable Agriculture.*

1. INTRODUCTION

Traditional agriculture plays an important part in fulfilling the food needs of a rising human population, but it has also resulted in a greater reliance on artificial fertilizers and pesticides. Chemical fertilizers are man-made chemicals with known amounts of nitrogen, phosphorous, and potassium, and their usage pollutes the air and ground water by causing eutrophication of water bodies. In this respect, current efforts have been focused on producing "nutrient-rich, high-quality food" in a sustainable manner to guarantee bio-safety. The increasing demand for biologically based organic fertilizers as an alternative to agrochemicals is attracted by a new approach to agricultural production. Encourage alternative soil fertilization methods that rely on organic inputs in agriculture to enhance nutrient availability and field management. Organic farming is one of these methods that contributes to soil biodiversity while also ensuring food safety. Bio-fertilizers also have a longer shelf life and have no negative environmental consequences.

Organic farming relies heavily on the soil's natural microflora, which includes a diverse range of beneficial bacteria and fungus, including arbuscular mycorrhiza fungi (AMF), often known as plant growth-promoting rhizobacteria (PGPR). By nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth regulating chemicals, antibiotic synthesis, and biodegradation of organic materials in the soil, biofertilizers maintain the soil environment rich in all types of micro- and macronutrients. When applied as seed or soil inoculants, biofertilizers proliferate, participate in nutrient cycling, and improve crop production. In average, 60% to 90% of all applied fertilizer is wasted, with the remaining 10% to 40% taken up by plants.

Microbial inoculants play a critical role in integrated nutrient management systems to maintain agricultural production and a healthy environment in this respect [1]–[3].

Fertilizers with PGPR or PGPR plus AMF co-inoculants may improve fertilizer nutrient utilization efficiency. 70 percent fertilizer with AMF and PGPR for P absorption was better adapted to a synergistic interaction of PGPR and AMF. On a whole-tissue basis, a similar pattern was seen in N absorption, with 75 percent, 80 percent, and 90 percent fertilizer + inoculants being substantially equivalent to 100 percent fertilizer. This review is aimed for agriculturists and plant biologists whose work focuses on developing clean and effective ways to enhance soil quality by feeding and preserving the beneficial and natural flora of microorganisms or PGPRs. It also discusses current advances in the field of field management, including the possible use of biofertilizers to enhance nutrient profiles, plant growth and production, and tolerance to environmental stress, with a focus on the mechanism of biofertilizers' success [4], [5].

The Microbiome

The rhizosphere, or narrow zone of soil around plant roots, may contain up to 10¹¹ microbial cells per gram of root and over 30,000 prokaryotic species, all of which help to boost plant production. The microbiome is the collective genome of the rhizosphere microbial community that surrounds plant roots and whose interactions determine crop health in natural agroecosystems by providing numerous services to crop plants such as organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling, weed control, and bio-control. Using 454 sequencing (Roche) of 16S rRNA gene amplicons, the metagenomics research provides the person with information about the core rhizosphere and endophytic microbiomes activity in *Arabidopsis thaliana*. It has been suggested that using tailored core microbiome transfer treatment in agriculture may be a viable strategy for controlling plant diseases in various crops. Rhizosphere microbial communities as a chemical fertilizer alternative have sparked a lot of interest in sustainable agriculture and bio-safety programs [6], [7].

In the next decades, a significant emphasis will be on using beneficial microorganisms in sustainable agricultural production to create safe and environmentally acceptable techniques. Inoculation of the soil ecosystem with such microorganisms improves soil physicochemical characteristics, soil microbial biodiversity, soil health, plant growth and development, and agricultural production. Plant growth-promoting rhizobacteria, N₂-fixing cyanobacteria, mycorrhiza, plant disease-suppressing beneficial bacteria, stress tolerance endophytes, and bio-degrading microorganisms are among the agriculturally important microbial communities. Biofertilizers are a supplement to soil and crop management practices such as crop rotation, organic adjustments, tillage maintenance, crop residue recycling, soil fertility renovation, and pathogen and insect pest biocontrol, all of which can help to ensure the long-term viability of various crop productions. Some of the PGPRs reported to grow in the soil under no tillage or minimal tillage treatments include *Azotobacter*, *Azospirillum*, *Rhizobium*, cyanobacteria, phosphorus and potassium solubilizing microorganisms, and mycorrhizae. Efficient strains of *Azotobacter*, *Azospirillum*, *Phosphobacter*, and *Rhizobacter* may supply large amounts of nitrogen to *Helianthus annuus*, allowing it to grow taller, have more leaves, have a higher percentage of seed filling, and have a higher seed dry weight. Similarly, adding *Azotobacter*, *Azospirillum*, and *Rhizobium* to rice enhances the physiology and root morphology.

Because it has a range of metabolic activities, Azotobacter plays an essential part in the nitrogen cycle in nature. Azotobacter may generate vitamins like thiamine and riboflavin, as well as plant hormones like indole acetic acid (IAA), gibberellins (GA), and cytokinins, in addition to nitrogen fixation (CK). Plant development is improved by *A. chroococcum*, which increases seed germination and advances root architecture by suppressing pathogenic bacteria surrounding crop root systems. *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. nigricans*, *A. armeniacus*, and *A. paspali* are among the species in this genus. Wheat, oat, barley mustard, seasmum, rice, linseeds, sunflower, castor, maize, sorghum, cotton, jute, sugar beets, tobacco, tea, coffee, rubber, and coconuts are among the crops for which it is used as a biofertilizer. *Azospirillum* is a free-living, motile, gram-variable, aerobic bacteria that thrives in flooded environments and supports plant growth and development in a variety of ways. *Azospirillum* has been proven to improve plant growth and agricultural production in both greenhouse and outdoor experiments. Various *Azospirillum* species, including *A. lipoferum*, *A. brasilense*, *A. amazonense*, *A. halopraeferens*, and *A. irakense*, have been found to increase agricultural production. Intriguingly, *Azospirillum* inoculation has been shown to alter root shape by generating plant growth controlling chemicals through siderophore synthesis. It also boosts the development of root hairs and increases the number of lateral roots, giving the roots greater surface area to absorb nutrients. This enhances the plant's water status and helps the nutritional profile in the growth and development of the plant. *Azospirillum brasilense* and *Rhizobium meliloti* plus 2, 4D co-inoculation had a favorable impact on grain production and N, P, and K content of *Triticum aestivum*. For many years, *Rhizobium* has been employed as an effective nitrogen fixer. By turning air nitrogen into useable forms, it serves a critical role in boosting yield. *Rhizobium* usually penetrates the root hairs, multiplies there, and produces nodules because it is resistant to a wide range of temperatures. *Rhizobium* inoculants were found to substantially enhance grain yields of Bengal gram, lentil, pea, alfalfa, and sugar beet rhizosphere, berseem, ground nut, and soybean in various locations and soil types. These *Rhizobium* isolates from wild rice have been shown to provide nitrogen to the rice plant, allowing it to grow and develop. *Sinorhizobium meliloti* 1021 is a *Rhizobium* species that infects plants other than leguminous plants, such as rice, to boost growth by increasing endogenous plant hormone levels and photosynthetic performance to impart stress tolerance. The IRC-6 *Rhizobium* strain has improved many important characteristics in groundnut, including the quantity of pink colored nodules, nitrate reductase activity, and leghaemoglobin concentration in 50 DAI (days after inoculation).

Crop Nutrient Profile and Bio-fertilizer Exploitation:

Beneficial microorganisms have the ability to absorb phosphorus for their own needs, which is then accessible in adequate amounts in soil as its soluble form. In the solubilisation process, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Fusarium*, *Sclerotium*, *Aspergillus*, and *Penicillium* have been shown to be active. *Micrococcus* sp. NII-0909, a phosphate-solubilizing bacterial strain, exhibits polyvalent characteristics such as phosphate solubilization and siderophore synthesis. Similarly, two fungus, *Aspergillus fumigatus* and *Aspergillus niger*, were isolated from decaying cassava peels and shown to convert cassava wastes to phosphate bio-fertilizers using a semi-solid fermentation method. Stress-tolerant bacteria *Burkholderia vietnamiensis* generate gluconic and 2-ketogluconic acids, which aid in phosphate solubilization. Isolated from the rhizosphere of sunflowers, *Enterobacter* and *Burkholderia* generate siderophores and indolic compounds (ICs) that may solubilize phosphate. Potassium solubilizing

microorganisms (KSM) of the genera *Aspergillus*, *Bacillus*, and *Clostridium* have been shown to be effective in solubilizing potassium in soil and mobilizing it in various crops. Mycorrhizal mutualistic symbiosis with plant roots meets plant nutritional demands, resulting in improved plant growth and development as well as protection from diseases and environmental stress. It causes phosphate to be absorbed by hyphae from the exterior and transferred to internal cortical mycelia, which then transport phosphate to the cortical root cells [55]. *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectonema* are examples of nitrogen-fixing cyanobacteria that are widely employed as biofertilizers. Apart from nitrogen, the growth-promoting compounds and vitamins released by these algae *Cylindrospermum musicola* boost rice plant root development and yield. Intriguingly, *Anabaena* sp. strain PCC7120's nitrogen-fixing ability was improved via genetic engineering. In *Anabaena* sp. strain PCC7120, constitutive expression of the *hetR* gene controlled by a light-inducible promoter increased *HetR* protein production, resulting in greater nitrogenase activity than the wild-type strain. As a result, when paddy was planted in the fields, it grew faster [4], [8].

The importance of biofertilizers and plant resistance to environmental stress:

The main limitations that are influencing agricultural production are abiotic and biotic stressors. Many contemporary scientific techniques have been widely used for crop development under stress, with PGPRs' function as bioprotectants becoming more important. Under salt stress, *Rhizobium trifolii* inoculated with *Trifolium alexandrinum* produced more biomass and had more nodulation. It has been shown that *Pseudomonas aeruginosa* can survive both biotic and abiotic stressors. Osmolytes and salt-stress induced proteins are produced by *P. fluorescens* MSP-393 to counteract the detrimental effects of salt. Under alkaline and high salt conditions, *P. putida* Rs-198 increased germination rate and many growth parameters such as plant height, fresh weight, and dry weight of cotton by increasing the rate of uptake of K^+ , Mg^{2+} , and Ca^{2+} while decreasing the absorption of Na^+ . Plant resistance was given by a few strains of *Pseudomonas* through 2,4-diacetylphloroglucinol (DAPG). *P. fluorescens* DAPG was discovered to elicit a systemic response against *P. syringae* in *Arabidopsis thaliana*. *P. alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium phlei* MbP18 generate calcisolin, which allows them to withstand high temperatures and salt stress. Plant inoculation with AM fungus has also been shown to enhance plant development under salt stress. Under 172 mM NaCl and water stress, *Achromobacter piechaudii* was also shown to enhance the biomass of tomato and pepper plants. A root endophytic fungus called *Piriformospora indica* was discovered to protect the host plant from salt stress. In one study, it was shown that inoculating *Lactuca sativa* with PGPR alone or in combination with AM such as *Glomus intraradices* or *G. mosseae* led in improved nutrient absorption and normal physiological processes in *Lactuca sativa* under stress circumstances.

Under salt stress, the same plant treated with *P. mendocina* produced more shoot biomass. Using transcriptomic and microscopic methods to investigate mechanisms involved in osmotic stress tolerance, researchers discovered a significant shift in the transcriptome of *Stenotrophomonas rhizophila* DSM14405T in response to salt stress. The bean plants were enabled to overcome drought stress by a combination of AM fungus and N_2 -fixing bacteria. Other crops that have been affected by *A. brasilense* and AM include tomato, maize, and cassava. The combination of *A. brasilense* and AM enhanced plant resistance to abiotic stressors. Drought stress was alleviated by combining the effects of *Pseudomonas putida* or *Bacillus megaterium* with AM fungus. The

antioxidant and photosynthetic pigments in basil plants were enhanced by using *Pseudomonas* sp. during water stress. Under water stress, a combination of three bacterial species produced the greatest CAT, GPX, and APX activity, as well as the maximum chlorophyll content in leaves. Under water stress, *Pseudomonas* spp. were shown to have a beneficial effect on seedling development and seed germination in *A. officinalis* L. After inoculating rice plants with arbuscular mycorrhiza, photosynthetic efficiency and antioxidative response increased in drought-stressed rice plants. Drought and salty environments have both been used to demonstrate the positive benefits of mycorrhizae. Heavy metals from hospital and industrial waste, such as cadmium, lead, and mercury, build in the soil and enter plants via the roots. *Azospirillum* spp., *Phosphobacteria* spp., and *Glucanacetobacter* spp. were shown to be more resistant to heavy metals, particularly iron, when isolated from the rhizosphere of rice fields and mangroves. Canola and barley plants are protected against the inhibitory effects of cadmium by *P. potida* strain 11 (P.p.11), *P. potida* strain 4 (P.p.4), and *P. fluorescens* strain 169 (P. f.169) through IAA, siderophore, and 1-aminocyclopropane-1-carboxylate deaminase (ACCD). It has been claimed that introducing microorganisms in the form of effective microbial agents (EMA) to plant species such as cotton, ryegrass, tall fescue, and alfalfa may speed up rhizoremediation of petroleum-contaminated soil [9]–[11].

2. DISCUSSION

The connection of fungus with the roots of higher plants is known as mycorrhiza. While it remains a mystery, it serves as a model system for understanding the process underlying mycorrhizal inhabitation's promotion of root cell development. Genome sequencing of two EM fungus (ectomycorrhizae), *L. bicolor* 13 and *T. melanosporum* (black truffle) 14, aids in the discovery of factors that control mycorrhizal growth and function in plant cells. In *L. bicolor*, fifteen genes that were upregulated during symbiosis were identified as potential hexose transporters. Its genome lacked invertase genes, leaving it reliant on plants for glucose. However, unlike *L. bicolor*, *melanosporum* has one invertase gene and may utilize the host's sucrose directly. The upregulation of transporter genes during symbiosis showed that beneficial chemicals such as amino acids, oligopeptides, and polyamines were transported from one creature to another via the symbiotic interface. Mycelium that is free to move around may absorb nitrate and ammonium from the soil. These chemicals subsequently make their way to the mantle and Hartig net, where they are transmitted to the plants. In the development of symbiotic interfaces, fungus cysteine-rich proteins (MISSP7) play an essential role as effectors and facilitators.

During mycorrhizal colonization, several genes involved in auxin production and root morphogenesis were up-regulated. *G. versiforme* also has inorganic phosphate (Pi) transporters on its hyphae, which aid in the direct absorption of phosphate from the soil, and *G. intraradice* has a glutamine synthase gene, which strengthens the possibility of nitrogen metabolism in fungal hyphae that can be transported to the plant later. Myc factors, which are identical to *Rhizobium*'s Nod factors, are thought to be produced by mycorrhiza and *Rhizobium* and detected by host roots to activate the signal transduction pathway or common symbiosis (SYM) pathway. There are several similarities between the processes that prepare plants for AM and *Rhizobium* infection. With the initial encounter with fungal hyphae, the common SYM pathway prepares the host plant to undergo molecular and anatomical changes. Calcium, through Ca²⁺ spiking in the nuclear area of root hairs, is thought to be the center of secondary messengers thus far. According to microarray

research, *Rhizobium leguminosarum* biovar *viciae* may activate different genes in plants such as pea, alfalfa, and sugar beet. IAA is produced by PGPR, which causes the formation of nitric oxide (NO), which serves as a second messenger to activate a complex signaling network that leads to enhanced root growth and development.

During entrance, the expression of ENOD11, as well as numerous other defense-related and root remodelling genes, increases. As a result, the development of a pre-penetration apparatus, or PPA, is possible. Though the biology underlying arbuscule formation is unclear, a gene called vapyrin causes arbuscule growth to slow down when it is taken out. Many additional genes are known to play a role in arbuscule production, including subtilisin protease 65, phosphate transporter 66, and two ABC transporters 67. Today, scientists often employ nitrogenfixation genes to produce transgenic plants that can fix atmospheric nitrogen. The stimulation of *nif* genes in nitrogen-fixing bacteria occurs in the rhizosphere at low nitrogen and oxygen concentrations. When compared to a *G. diazotrophicus* mutant with a mutant *nif D* gene, sugarcane plantlets inoculated with a wild strain of *G. diazotrophicus* showed fixation of radioactive N₂, demonstrating the importance of *nif* genes. The efficiency of nitrogen fixing is determined by carbon consumption. When a bacteria like *Bacillus subtilis* (UFLA285) is given to a cotton plant, it may stimulate 247 genes differently than when no PGPR is given to the cotton plant.

3. CONCLUSION

Environmental stress is becoming a significant issue, and productivity is dropping at an alarming pace. Our reliance on chemical fertilizers and pesticides has aided the growth of businesses that produce life-threatening substances that are not only harmful to human health but also have the potential to disrupt the ecological balance. Biofertilizers may assist in solving the issue of feeding an ever-increasing global population at a time when agriculture is under pressure from a variety of environmental factors. It is critical to recognize the benefits of biofertilizers and to apply them to current agricultural methods. The new technique, which was created utilizing the strong instrument of molecular biotechnology, has the potential to improve the biological pathways for phytohormone synthesis. These technologies may assist offer alleviation from environmental stressors if they are discovered and transferred to useful PGPRs. However, one of the few reasons why many beneficial PGPRs are still outside the understanding of ecologists and agriculturists is a lack of information about better biofertilizer administration methods in the field. Nonetheless, recent advances in microbial research, plant-pathogen interactions, and genomics will aid in the optimization of the necessary procedures. The success of biofertilizer research is dependent on the development of novel methods linked to the functions of PGPRs and their appropriate use in agriculture. The main difficulty in this field of study is that, in addition to identifying different strains of PGPRs and their characteristics, it is also necessary to dissect the real mechanism of PGPRs' working for their effectiveness in sustainable agriculture.

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