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An Overview: Mechanism Involved in Bio-Priming Mediated Plant Growth Promotion

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ABSTRACT

Application of plant growth promoting rhizho-bacteria in agriculture is a need of time for sustainable agriculture. Bio-priming is a treatment of seeds with beneficial microorganism under controlled hydration which enhances the preparatory processes prior to germination without the emergence of the radicle. It improves the seedling vigor, germination percentage, speed of germination, growth and development. Bio-priming agents directly release growth promoting hormones viz., indole acetic acid, gibberellin, and cytokinin or stimulate their production in the plant, they also improve the availability of minerals viz., nitrogen, phosphorous, potassium, and Iron whereby enhance the plant growth and development. Bio-priming affects plant by different indirect means viz., phytohormones production stimulation, modulation in different secondary metabolites, alteration in gene expression, tolerance to abiotic and biotic stress. The PGPR bio-priming enhances production of soluble protein, soluble sugar, phenolic acid, salicylic acid and plant growth hormones. Further, efficient mitochondrial development by augmenting energy metabolism is a specialty of bio-primed seeds. The bio-priming also found to induce early DNA and protein synthesis. Bio-priming reported to enhance the expression of RuBisCO and chl a/b, expansins, β-tubulin and GST genes which at the end leads to have better carboxylation capacity and efficient photosynthesis whereby stimulate early germination process and vigorous plant growth. Current biochemical and molecular level understanding confirms the beneficial effect of bio-priming on seed germination, plant stand establishment, growth and development.

Key words: *Bio-priming, PGPR, Physiology, Biochemical, Gene expression*

INTRODUCTION

The agriculture sector contributes 7.8 percent to Indian economy which is much higher than the world average i.e., 6.1 percent[1]. Increasing population and awareness of health leads to huge pressure for quality adequate foods at a correct time and that should be free from an unacceptable level of chemicals. To accomplish this goal, every country switched over to organic farming or sustainable farming. Good quality seeds with rapid germination, producing synchronized vigorous seedlings, higher yield potential and productivity are very important factors for agriculture.

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Seed is preferred technique of agriculture scientist for disseminating any technology as it is easy means of adaptation and cost-effective.

Secretion of nutrients by plant roots enhances the abundance of microorganisms in the rhizosphere[2]. Plant and rhizospheric microorganism have a close association, some are beneficial and others are detrimental. Beneficial microorganisms have the ability to live within or in the vicinity of plant roots and promote plant growth and development are known as plant growth promoting rhizobacteria (PGPR) [3,4]. The direct and indirect effects of PGPR on plant growth and development are noticeable[5,6]. Besides the promotion of plant growth, it possesses the ability to suppress soil born pathogens by producing antibiotics[7] and by induced systemic resistance[8,9]. It also strengthens plants to tolerate under abiotic stress conditions like salt and drought[10,11,12], chilling stress[13] etc. Further, the PGPR plays an important role in improving and maintaining soil structure and bioremediation of contaminated soil[14]. The PGPR based on the application in agriculture are classified into various groups (Table 1).

Application of PGPR in the agriculture is a potential alternative to chemical-based agriculture which had severely destroyed the agro-ecosystem[34]. The first biological product based on *Bacillus subtilis was* bacteriological fertilizer for inoculation of cereals, marketed in 1897 under the proprietary name "Alinit" by Farbenfabriken[35]. For a sustainable agriculture, symbiotic PGPR (*Rhizobium* and *Frankia*) and non-symbiotic PGPR (*Azatobacter*, *Azospirillum*, *Bacillus* and *Klebsiella*) are commercially applied to enhance yield and reduce the use of harmful agrochemicals[36]. Survival of PGPR on seed and in soil mainly depends on the method of application and competency of the introduced bacteria with other bacteria present in the rhizosphere. Augmentation of PGPR into plants can be achieved by different methodologies including direct soil application, root dipping method, seed coat pelleting and seed priming. Direct application of inoculums into the soil is advisable when the plant tissue contains some antagonist microbes or pesticidal compounds[37]. It is a simple and easy method but the requirement of large inoculums make it very costly further, it needs special care during transportation and after field application[38]. The root dipping method is mainly focused for biocontrol but it requires preparation of plant nurseries which is not cost effective for some plants [39,40,41].

Seed pelleting hinders gaseous exchange whereby reduce nitrogen fixation in leguminous seeds so seed treatment is considering as a most effective alternative technique as it required only small dose of microbial inoculum with high efficiency[42]. Seed priming is a modern seed treatment method involves soaking of seeds in a solution of a specific priming agent with restricted water availability under controlled conditions followed by drying of seeds into its original weight that initiates preparatory germination related process[43] and maintaining the seeds in phase II stage of imbibition by extending lag phase of germination[44]. Different seed priming methods are available which are depending on the substances used named as hydro priming, halo priming, osmopriming, hormonal priming and bio-priming. Hydro-priming means seed are soaking in water[45]. Inorganic salts like NaCl, KNO_3, $CaCl_2$ and $CaSO_4$ etc. are used in halo priming[46,47]. The optimum concentration of plant growth hormones like auxin, gibberellin, abscisic acid and ethylene have been used in hormonal priming[48,49,50]. The osmopriming means priming with osmoticants like sugar, mannitol, polyethylene glycol (PEG) etc.[51,52,53] and bio-priming means the use of beneficial microorganisms[54,55]. This review mainly emphasized on physiological, biochemical and molecular changes in seedling of PGPR bio-primed seeds and how these changes are influencing germination, seedling vigor, growth, development and productivity of plants.

BIO-PRIMING

Bio-priming is an emerging trend aimed to improve seed quality, seedling vigor, productivity and resistance to biotic and abiotic stress by reducing the use of chemical inputs for sustainable agriculture. Bio-priming is a treatment of seed with beneficial microorganism under controlled hydration which enhances the preparatory processes prior to germination without the emergence of the radicle. It is associated with an increase in hydrolytic enzyme activities, reactive oxygen species (ROS) detoxifying enzymes activities and alteration in internal plant hormone levels, and also a differential expression of genes in plants that contributes the enhanced plant growth and resistance against biotic and abiotic stress. Innovative research studies at biochemical, proteomics and transcriptome levels are necessary to understand the role of bio-priming with PGPRs in phyto-stimulation and nutrient enhancement.

The direct effect of Bio-priming

Plant growth promoting rhizobacteria in primed seeds are colonizing the root surface and competing with other microorganisms in the rhizosphere. Bio-priming is directly involved in the enhancement of plant growth by the secretion of compounds and mineral solubilization. Nitrogen is an important constituent of amino acid, chlorophyll and other structural components of plants[56]. Nitrogen contributes dark green color to plants, promote leaves, stems and other vegetative growth and improve fruit quality in plants[57]. PGPR in the primed seeds converts atmospheric nitrogen into plant absorbable form by the process of biological nitrogen fixation (BNF) due to the presence of nitrogenase which is coded by *nif* gene[58,59]. Glick *et al*.,[60] and Ahemad and khan,[61] reported that nitrogen-fixing PGPR may be symbiotic like *Diazotrophs* (forms non-obligated interaction) and *Rhizobia* (forms nodules) and non-symbiotic like *Azospirillum*, *Azotobacter* and *Cyanobacteria* etc. A major portion of soil phosphorus is in insoluble form[62], phosphorus solubilising microorganism (PSM) like *Azotobacter, Bacillus, Beijerinckia, Enterobacter, Microbacterium, Pseudomonas and Serratia* release complex or mineral dissolved compounds like organic anions, protons, hydroxyl ions, carbon dioxide or liberation of extracellular enzymes namely phosphatase leads to solubilize the phosphorus and make it available to plant[63,64]. Phosphorous is an essential part of nucleic acids, phosphoproteins, phospholipids, energy-rich phosphate molecules and enzymes in plants. Phosphorous influences the lateral root morphology, root development, root branching and root to shoot ratio[65,66]. Some PGPR used in bio-priming have the ability to solubilize potassium from potassium-bearing minerals (Mica, illite and Orthoclase) by excretion of organic acids (citric acid, tartaric acid and oxalic acid) directly dissolving the rock potassium or chelate the silicon ion[67]. Potassium improves nitrogen use efficiency, involved in enzyme activation, stomatal activity, water and nutrient transport, transport of sugar and photosynthesis in the plant[68,69,70,71]. Siderophore producing rhizobacteria improves iron uptake in oats and *Arabidopsis*[72,73].

Siderophore production by bio inoculant in primed seeds has suppressed the disease and improved plant growth[74,75]. It forms a stable complex with other heavy metals like Al, Cd, Cu, In, Pb and Zn which are an environmental concern[76,77]. Some of the PGPR like *Pseudomonas putida*, *Bacillus* sp. and *Enterobacter* sp. etc. are able to produce 1-Amino cyclopropane-1- carboxylate (ACC) deaminase under stress condition and showed reduction in ethylene content in plant whereby facilitated its growth and development[78]. Bio-priming of wheat with rhizobacteria containing ACC deaminase activity increased the growth and yield under stress condition[79]. Pattern and Glick[80], reported that PGPR possesses the ability of synthesis and release of auxin which is an enhancer of plant cell division and differentiation, xylem and root development, and initiation of lateral and adventitious root formation. Bio-priming of *Salvia officinalis* with PGPR having the ability to produce moderate auxin, recorded high germination rate, reduced speed of germination and higher root growth[81]. Glick[64], reported bio-priming with IAA releasing rhizobacteria loosened plant cell walls and increased the root exudation from the plant which enhanced the availability of additional nutrients to the microorganism and supported its growth in the rhizosphere. The overproduction of ACC due to bio-priming with IAA producing microorganisms negatively regulated the root growth[82]. The gibberellin and cytokinin produced by *Azotobacter chroococcum* and *Rhizobium leguminosarum* have a positive effect on plant height and growth[19, 21].

Effect on physiological characters

Root is a key nutrient feeder to plant, further quality root biomass leads to better plant productivity. Root architecture (root length, root depth, root thickness and root to shoot ratio etc.) are playing an important role in strengthening the plant to survive in water deficit environment[83]. Seed bio-priming with PGPR reported to enhance the root related traits and producing a vigorous root system. Marcela *et al*., [84] reported an increased root to leaves ratio in bio-primed rice. High root to shoot ratio, root length, root dry weight increased leaves number, leaf area and chlorophyll content have reported in bio-primed crops[85,86]. Anitha *et al*.,[87] reported increased root length and shoot length in soybean after bio-priming. Marcela *et al.*[84] reported increased lignin content in PGPR treated rice. Lignin involved in the better organization of macro fibrils which mediate the structural stability of the root cell wall[88]. Plant growth, nutrient uptake and nutrient use efficiency, synchronisation in germination and vigorous plant growth, speed of germination, and good plant stand under normal and stressed condition have been increased by seed bio-priming[89,90,91,92]. In bio-primed crops increased biomass production grain yield and productivity were the evidence of growth stimulation[89,93,94,95]. Bio-priming is a better solution for enhanced growth and development of micro-propagated plants which have reduced photosynthetic activity, poorly functioning stomata and underdeveloped root and shoot system[96]. Bio-priming with PGPR minimizing the time required for lignification of micro-propagated plants and accelerates production process[96,97].

Effects on biochemical parameters

In bio-priming, seeds are exposed to restricted water under controlled conditions which leads to the solute accumulation in the embryo and there will not be any germination till the embryo water potential reaches the threshold level required for radical emergence[98]. Bio-primed seeds have an advantage over non-primed seeds at the early process of germination because bio-primed seeds have large carbohydrate storage reserves which strengthen the plant to survive from low oxygen stress under flooded condition[99].

Bio-priming leads to biochemical changes *viz.*, enhanced production of proteins, hormones, phenol and flavonoid compounds contribute to better plant growth and development performance. Growth responses in herbaceous plants are determined by nitrogen reserve compounds like nitrates, amino acids and proteins[100,101]. Soluble protein percentage in bio-primed seeds and seedlings were higher compared to non-primed[102]. There was gain in total protein contentand free amino acid content during the different growth stages after bio-priming with PGPR[103,104,105]. Soluble sugars act as osmolytes to maintain cell homeostasis, further by regulating the signals and acting as primary messenger it plays an important role in the expression of different genes responsible for plant growth and metabolism in source and sink tissues[106,107,108]. Total soluble sugar and reduced sugar content in plant increased after bio-priming[109]. Efficient mitochondrial development by augmenting energy metabolism *i.e.* high ATP pool and an efficient ATP producing system occurs due to early imbibition process in primed seeds[110]. PGPR used in bio-priming enhanced the synthesis of specific phenolic acid in plants at different growth stages[111]. The combined application of PGPR increases the total phenol content in rice[84]. Increased in indole acetic acid content in the plant by bio-priming enhanced number of roots, root hairs and root area, plant cell division, cell differentiation, xylem and root development, lateral and adventitious root formation, pigment formation and biosynthesis of variable metabolites[112]. Besides plant growth promotion, bio-priming also stimulate the production of defense-related enzymes (peroxidase, superoxide dismutase, catalase, chitinase, ammonia lyases, etc.) which offer fitness benefit to plants against biotic and abiotic stress.

Effects on gene expression

Transcript level expression modulation is a key to a living system for response. Bio-priming leads to transcriptome level changes that make plant quick responsive. Microtubules consist of α-tubulins and β-tubulins, which are the structural elements in cell growth and morphogenesis, and also involved in regulation and signal transduction[113]. Accumulation of β-tubulin preceded DNA replication and in primed seeds, the induction of DNA synthesis was started earlier, approximately 12 h earlier than untreated seeds. Amount of induced nuclear replication activity and β-tubulin was higher after priming in pepper and were found to be correlated with improved seed germination[114]. The transcriptome level studies provide a platform for comparative analysis of the plant roots response towards PGPR used in bio-priming and a pathogen. Repairing of the damaged part of the seeds and reduction in metabolic exudates were facilitated with priming by early transcription and protein synthesis[115]. A transcriptome level study of *Bacillus subtilis* was done by Xie *et al*., [116] in response to interaction with rice. According to that study, 176 genes showed a significant change in expression level in bacteria. Among these, 52 up-regulated genes were involved in metabolism and transport of nutrients and stress responsive where as 24 genes were down-regulated and involved in chemotaxis, motility, sporulation and teichuronic acid biosynthesis. Priming of rice plant with *Pseudomonas fluorescence* showed over expression of *RuBisCO*, led to increased photosynthesis activity[117]. Priming enhanced the expression of *RuBisCO* and *chl a/b* binding genes which increased carboxylation capacity and photosynthesis efficiency[118,119]. The respiration, energy metabolism and early reserve mobilization events in crops were regulated by bio-priming[110,120]. Up-regulation of expansin gene which is responsible for cell wall loosening was found important for coleoptile elongation[121,122]. The loosening of cell due to expansin occurs by disrupting hydrogen bonds between cellulose microfibrils and matrix polymers[123]. Enhanced expression of expansin genes was reported by Hussain *et al*.,[118] in bio-primed rice plant under submerged condition. Further, the enhanced expression of *NADH-GOGAT* and *GAPDH*, involved in energy production and biomass accumulation were reported in rice[124,118]. Putative glutathione s-transferase (*GST*) gene, an auxin-inducible gene which recognizes and transfers broad spectrum of reactive electrophilic compounds produced exogenously and endogenously to the vacuole by glutathione pump[124,125]. Kandasamy *et al.*,[117] found that overexpression of *GST* in rice plant primed with *Pseudomonas fluorescence* have an essential role in induced systemic resistance (ISR) and protecting cells from oxidative damage. Bio-priming also induced overexpression of genes involved in the regulation of secondary metabolism, development, transport protein and metal handling.

Table 1: Major groups of PGPR based on the application in agriculture

Group	Application	PGPR	Reference
Bio-fertilizer	Increasing the availability of nutrients to plant	*Azotobacter chroococcum*	Kumar *et al.*, [15]
		Gluconacetobacter diazotrophis	Saravanan *et al.*, [16]
		Bacillus sp.	Canbolat *et al.*, [17]
		Stenotrophomonas maltophilia	Mehnaz *et al.*,[18]
Phyto stimulator	Plant growth promotion generally through phyto hormones	*Rhizobium leguminosarum*	Noel *et al.*, [19]
		Bradiyrhizobium sp.	Antoun *et al.*, [20]
		Azotobacter chroococcum	Verma *et al.*,[21]
		Xanthomonas sp.	Sheng and Xia, [22]
		Rhizobium phaseoli	Zahir *et al.*,[23]
		Bacillus sp.	Wani and Khan,[24]
Rhizo remediation	Degrading organic pollutant	*Kluyvera ascorbata*	Genrich *et al.*, [25]
		Pseudomonas putida	Tripathi *et al.*, [26]
		Bradyrhizobium sp.	Dary *et al.*, [27]
		Psychrobacter sp.	Ma *et al.*, [28]
Biopesticide	Controlling diseases mainly by producing antibiotics and antifungal metabolites	*Pseudomonas chlororaphis*	Liu *et al.*, [29]
		Bacillus subtilis	Cazorla *et al.*, [30]
		Pseudomonas fluorescens	Braud *et al.*, [31]
		Streptomyces sp.	Bhattacharyya and Jha, [32]
		Micromonospora sp.	Franco-correa *et al.*, [33]

CONCLUSION

Bio-priming has a direct and indirect effect on plant growth and development. PGPR used in bio-priming have the ability to produce plant growth hormones (indole acetic acid, gibberellin and cytokinin), also have nutrient solubilization (phosphate, zinc and potassium) and nitrogen fixation ability which help plant for better growth and development. Further, bio-priming also act as a biocontrol agents producing some antibiotics, antifungal metabolites and by degrading organic pollutants. Bio-priming has reported a positive impact on seedling vigour, germination percentage, speed of germination, growth, development, yield and productivity of crops. Bio-priming with PGPR enhances the root related traits and producing vigorous plant root system, which is key for better production. Bio-priming also improve the total soluble sugar, reduced sugar content, total protein content and ATP production in plant that leads to optimum plant growth. Efficient mitochondrial development by augmenting energy metabolism is a speciality of bio-primed seed. Induction of DNA synthesis, transcription and protein synthesis starts earlier in primed seeds than the non-primed seeds. Further, bio-priming found to enhance the expression of *RuBisCO*, *chl a/b* protein-coding genes, expansins genes, *GST* gene and β-tubulin gene whereby positively modulate the plant transcriptome for better growth, development and tolerance against biotic and abiotic stress. Bio-priming improves overall plant growth and development by physiological, biochemical and molecular level alteration which at the end resulted in to an asset to the modern agriculture.

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