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Jagjot Kaur
Department of Microbiology,
Punjab Agricultural University,
Ludhiana, Punjab, India

Madhurama Gangwar
Department of Microbiology,
Punjab Agricultural University,
Ludhiana, Punjab, India

Gulab Pandove
Punjab Agricultural University,
Regional Research Station,
Bathinda, Punjab, India

Correspondence
Jagjot Kaur
Department of Microbiology,
Punjab Agricultural University,
Ludhiana, Punjab, India

Mitigating the impact of climate change by use of microbial inoculants

Jagjot Kaur, Gulab Pandove and Madhurama Gangwar

Abstract

According to world guesstimates, abiotic factors leads to an average of 50% yield losses in agricultural crops like high temperature (20%), low temperature (7%), salinity (10%), drought (9%) and other forms of stresses (4%). The implication of plant growth promoting rhizobacteria (PGPR) to conflict the harmful effects of ecological stresses and enhance plant growth and productivity by direct and indirect mechanisms has been reported. Plant growth promoting rhizobacteria (PGPR) live in association with roots of plant, elicit the largest influence on plants, immunity and affecting their productivity. PGPR can facilitate plant growth indirectly by reducing plant pathogens or directly by influencing phytohormone production (e.g. auxin, gibberallin or cytokinin), by facilitating the uptake of nutrients from the environment, and/or by lowering the levels of plant ethylene enymatically, nitrogen fixation, mineral phosphate solubilization (MPS), sequestration of iron by secretion of siderophores by release of volatiles. PGPR are now being used worldwide as bio-inoculants/biofertilizers to promote plant growth and development. Microbial inoculants could play an important role in stress management in the edaphic stress prone areas. Thus adaptability of microbial inoculants over wide range of pH, temperature and salt concentration is crucial for their application under different agro climatic conditions. Peat is the most successively used carrier because of high surface area and high water holding capacity. Peat based carriers are not easy to use with sophisticated planting equipment. Liquid inoculants can be easily adapted to advanced seeding equipment. This review will overview the various mechanisms by which microbial inoculants could mitigate the impact of climate change.

Keywords: Abiotic, PGPR, phytohormone, microbial, inoculants, edaphic, stress

1. Introduction

Variability in climate is one of the biggest environmental threats to agriculture peculiarly to wheat crop [1].

Increased concentrations of greenhouse gases have eventualized in increased temperature, evaporation, increased ambiguity of monsoon rainfall, increased recurrence of extreme like floods, droughts, heat waves etc. All these biotic and abiotic stresses have profound impact on crop yield [2]. Soil erosion and desertification are other crucial ecological concerns affecting the degradation and loss of productive agricultural land. According to world guesstimates, abiotic factors leads to an average of 50% yield losses in agricultural crops like high temperature (20%), low temperature (7%), salinity (10%), drought (9%) and other forms of stresses (4%) [3]. Patil *et al* [4] also reported the substantial influence of temperature on the plant metabolic processes and thus on wheat quantity and quality. Stress tolerant crop varieties development through plant breeding and genetic engineering is essential but costly and a long drawn process, whereas microbial inoculation could be a more cost effective and environmental friendly option to ameliorate stresses in plants in a shorter time frame [5]. In agriculture the use of microbial inoculants has greatly increased during the past two decades [6] as both public and private sector agricultural research and development communities are working for solutions to problems associated with modern agriculture. Microbial inoculants mainly include fungi, arbuscular mycorrhizal fungi (AMF) and free-living bacteria [7]. Investigations have shown that certain species and/or strains of microbes strengthen the tolerance of plant to abiotic stresses such as excess salinity, nutrient deficiency or drought [8]. Plant growth promoting rhizobacteria have greatly impact the tolerance of agricultural plants to biotic and abiotic stresses (Figure 1). Plant growth promoting rhizobacteria (PGPR) live in association with roots of plant elicit the largest influence on plants, immunity and affecting their productivity. PGPR can facilitate plant growth indirectly by reducing plant pathogens or directly by influencing phytohormone production (e.g. auxin, gibberallin or cytokinin), by facilitating the uptake of nutrients from the environment, and/or by lowering the levels of plant ethylene enymatically [9], nitrogen fixation, mineral phosphate solubilization (MPS), sequestration of iron by secretion of siderophores by release of volatiles[10]. Yang *et al* [8] introduced the term 'induced systematic tolerance' (IST) that is caused by PGPRs. Induced systematic tolerance (IST) causes physical and chemical changes in plant, which results in tolerance to abiotic stresses. In view of these above explanation appropriate literature pertaining to various aspects have been reviewed under the following headings:

Heat stress

Heat stress is a function of rate and magnitude of temperature increase as well as the duration of exposure to the raised temperature [11]. Heat stress rationalizes biochemical, molecular and physiological changes that affect yield and quality of crop [12]. Heat stress also induces the of reactive oxygen species (ROS) production which destroys membranes and other systems of cells and trigger stress responses [13]. Reduction in yield induced by heat has been documented in many crops including cereals (e.g wheat, rice, maize, barley, sorghum,), pulse (e.g., chickpea, cowpea) and oil yielding crops (canola, mustard) [14-16]. In most parts of India, a sudden rise in temperature during grain filing prior to maturity proceeds in considerable reduction in yield. The duration of grain filling in cereals is determined principally by temperature.

Wheat grown under late planted conditions is exposed to very low temperature up to booting stage and face higher temperature at later stages that inhibits grain development, resulting into poor grain yield. The high temperature stress more prominent effects reproductive development than vegetative growth. Decline in yield with temperature is mainly associated with pollen infertility [17]. Exposure to heat stress in wheat crop accelerates the development stages to such a degree which cannot be paced by necessary supply of environmental inputs (radiation, water and nutrient) [18].

Heat stress affect the yield of wheat by causing reduction in tiller number, duration of grain filling phase, biomass, kernel size etc. Mitra and Bhatia[19] also reported in reduction in number of tillers, plant height and total biomass in rice cultivar in response to high temperature. Reduced number of tillers with promoted shoot elongation was observed in wheat plant under heat stress by Kumar *et al* [20]. Hundal [21] reported 15-17 per cent decrease in grain yield of wheat and rice by 20 ^{0}C increase in temperature and beyond that the decrease was very high in wheat.

Fluctuation in temperature could significantly influence the growth of plants by hormonal imbalances. Increase in ethylene production under temperature abuse condition been reported in plant tissues. Thus, an introduction of ACC deaminase containing rhizobacteria into the ecosystem of plant could ease unfavorable conditions caused due to temperature variations. ACC deaminase activity possessing rhizobacterium, *Burkholderia phytofirmans* inoculated in potato was able to maintain shoot and root biomass, stem length under temperature stress. The seed inoculation with beneficial bacteria seems a promising strategy to improve heat tolerance of wheat as disclosed by Abd-El-Daim *et al* [22].

Thus microorganisms seem a useful biotechnological tool in agriculture to ameliorate the negative effect of heat stress on crop plants [23]. However, concerning improvement of tolerance of heat stress using bacterial priming approaches very little information is available [22].

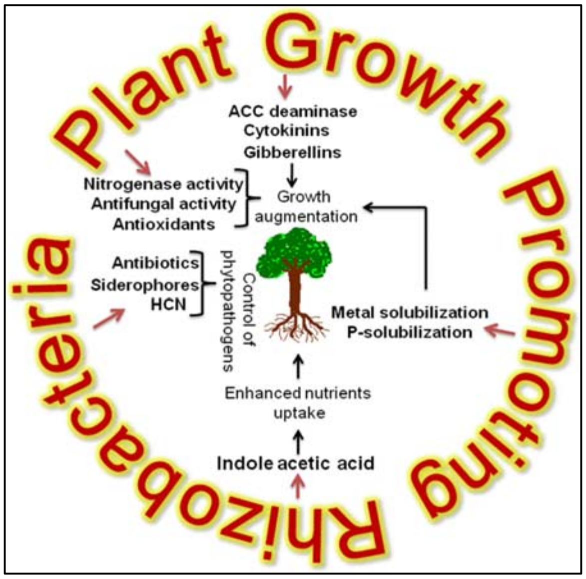

Fig 1: Mechanisms of plant growth promotion by rhizobacteria (Adapted from Ahemad and Kibret [24])

Sowing dates

Management in sowing time is one of the most paramount agronomic practices to counteract the adverse effect of temperature stress. Selection of optimum planting time diverts the high temperature stress during anthesis and grain filling. Sowing at optimum time reinforced germination, plant height, number of spikelets, grains spike and 1000-grain weight [25].

Tahir *et al* [26] reported maximum grain yield at proper sowing date and lower grain yield in late sowing. Tillering period reduces with late planting and hot weather during critical period of grain filling also reduces the grain yield due to forced maturity. Delay in sowing date of wheat leads to reduced wheat yield due to high temperature exposure, which reduce season length [27]. At tillering stage, optimum planting date could produce good crop growth that increases the cold tolerance [28]. Yawinder *et al* [29] and Bashir *et al* [30] stated that thousand grain weight decreased gradually with delay in planting time.

Jat *et al* [31] reported that the crop planted on 20th November achieved maximum height, dry-matter accumulation plant^{-1} and number of tillers than rest of sowing dates due to the availability of maximum growing period length than other dates.

Sardana *et al* [32] also reported that timely seeded crop produced maximum spike-bearing tillers m^{-2}, grains per spike and 1000-grain weight. In case of delayed planted crop, flowering stage and grain filling stage get coincide with rise in temperature and atmospheric drought during March and April, results in poor growth and low grain yield. Too early sowing produces weak plant with poor root system, which leads to intermittent death of the embryo, decomposition of endosperm due to activities of bacteria or fungi and irregular germination [33].

Salt stress

Salinity is another one of the grave environmental constraints in arid and semiarid regions of the world due to transgression of agricultural practices. Nearly 40 % of world's surface has salinity problems [34].

One possible strategy to counteract the adverse effect of salinity is to exploit the avenues of bio-agents or bio-inoculants [35]. Inoculation with PGPR, including strains of *Bacillus atrophaeus, Bacillus spharicus, B. subtilis* and *Staphylococcus kloosii*, increases nutrient content, chlorophyll content and yield of strawberry (*Fragaria ananassa*) plants under high saline soils conditions [36]. Soil fertility improved through introduction of salt-tolerant microorganisms which involves in decomposition of organic matter by nutrient cycling, by atmospheric nitrogen fixation and through growth hormones production [37].

Aly *et al* [38] stated that under NaCl conditions the application of *Azotobacter chroococcum* and/or *Streptomyces niveus* to maize plants influenced the content of DNA and RNA in shoots and roots, total soluble sugars total soluble proteins, total free amino acids, proline thus resulting in a higher salt tolerance of the plants. Hamdia *et al* [39] found an increase in total soluble and sacchrides, soluble protein in shoots and roots under salinity stress with *Azospirillum* inoculation of two maize cultivars. Increased growth and development of wheat plant reported by Sadeghi *et al* [40] in normal and saline conditions with *Streptomyces* C treated of soil.

Sadeghi *et al* [40] demonstrated that a *Streptomyces* isolate increased plant growth in wheat in presence of salt through production of auxin and indole acetic acid. Upadhyay *et al* [41] also showed that an increased total soluble sugar and proline in the wheat plants treated with PGPR significantly contributed to their osmotolerance. Inoculation of wheat seedlings with bacteria that produce exopolysaccharates (EPS) affect the restriction of stimulation and sodium uptake of plant growth under stress conditions caused by high salinity [5].

Mitigating strategies by use of microbial inoculants

Extensive research is being carried out, to develop tactics to tangle with abiotic stresses through shifting the crop calendars, resource management practices and development of heat and drought tolerant varieties[42]. The implication of plant growth promoting rhizobacteria (PGPR) to conflict the harmful effects of ecological stresses and enhance plant growth and productivity by direct and indirect mechanisms has been reported. According to Grover *et al.* [5], certain microbial types may mitigate the impact of soil drought through production of exopolysaccharates, proline, indole-acetic acid, induction of resistance genes and increased circulation of water in the plant and the synthesis of ACC-deaminase. Most bacterial strains investigated belong to the genera *Azotobacter*, *Bacillus*, *Pseudomonas* and *Azospirillum* and some are members of the Enterobacteriaceae [43]. These bacterial strains in plants stimulate the length of root hairs and density, the rate of appearance of lateral roots, root surface area, improve plant growth under water stress conditions, fix atmospheric nitrogen, solubilize inorganic phosphates and produce growth regulators [44].

The microorganisms which are beneficial can be significant component of management practices to achieve the significant yield which has been defined as crop yield limited only by the natural physical environment of the crop and its innate genetic potential [45].

Phytohormones produced by microbial inoculants

Phtytohormones play a vital role in ameliorating the biotic and abiotic stress due to climatic change. Plant development is regulated and systematized by activity of several phytohormones like abscisic acid (ABA), gibberellins (GAs), ethylene, auxin indole-3-acetic acid (IAA), cytokinins (CKs) and brassinosteroids (BRs) which control many physiological and biochemical processes in the sessile plant. These hormones may act either close to or remote from their sites of synthesis to regulate feedback to environmental stimuli or genetically programmed developmental changes [46]. There are number of reports of production of phytohormones by biofertilizer. Biofertilizers are plant growth promoting rhizobacteria (PGPR) contain strains from genera such *as Pseudomonas, Serratia, Azospirillium, Azotobacter, Bacillus, Burkholderia, Enterobacter, Rhi- zobium, Erwinia, Acinetobacter, Alcaligenes, Arthrobacter and Flavobacterium* [47], *Azotobacterium*, *Klebsiella*, *Xanthomonas* etc.[48] Production of indole acetic acid and gibberellins by PGPR, results in increased root length, root surface area and number of root tips, leading to enhanced uptake of nutrients thereby elaborating plant health under stress conditions.

Azotobacter has also reported to have beneficial effects on plant yields, due to their ability of fixing nitrogen [49], solubilizing phosphates [50] and produce phytohormones, like gibberellins, auxins and cytokinins [6, 50] vitamins like thiamine and riboflavin [51].

Actinomycetes, as other group of microorganisms, that may also bolster plant growth by the production of phytohormone-like compounds [52]. Some endophytic actinomycetes reported to have positive effects on host plants by production of plant growth regulators [53]. The PGP potential of *Streptomyces* sp. has been demonstrated on tomato, wheat, rice, bean and pea by Gopalakrishnan *et al* [54]. Several *Streptomyces* species, such as *S. olivaceoviridis*, *S. rimosus* and *S. viridis*, have the ability to produce IAA and thus improve plant growth by increasing seed germination, root elongation and root dry weight [55].

IAA Production

Indole-3-acetic acid (IAA) is the main member of the auxins family, it controls many important physiological processes like cell enlargement, cell division, tissue differentiation, responses to light and gravity [56]. Production of IAA by microbial isolates varies greatly among different species, strains of the same species, by culture condition, growth stage and availability of the substrates [57]. An important molecule that alters the level of IAA synthesis is the amino acid tryptophan, identified as the main precursor for IAA and thus plays a role in modulating the level of IAA biosynthesis [58]. Two major pathways have been proposed for IAA biosynthesis, the tryptophan-independent and tryptophan-dependent pathways [59].

PGPR produce IAA which induces the production of Nitric Oxide (NO), which acts as a second messenger to trigger a complex signaling network leading to improved root growth and developmental processes [60]. Under salinity or heavy metals stresses, IAA is reported to increase root as well as shoot growth of plants [61]. Inoculation of wheat with PGPRs producing IAA stimulated plant growth in normal and saline conditions [62]. Rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that implements additional nutrients to support the growth of rhizosphere bacteria [63].

It has been reported that inoculation with auxin-releasing *Azotobacter* strains increases growth, yield and nitrogen uptake in wheat and maize [64]. Several *Streptomyces* species, such as *S. olivaceoviridis*, *S. rimosus*, *S. rochei* and *Streptomyces* spp. from the tomato rhizosphere, have the ability to produce IAA and improve plant growth by increased seed germination, root elongation and root dry weight[62]. *Streptomyces* spp. also reported to increase the growth of wheat and legume (*Pisum sativum*) due to its ability to produce IAA [62].

Gibberallins (GA)

Gibberallic acid has been reported to alleviate the detrimental effects of environmental stresses on plant water relations [65]. Gibberellins are recognized to be a component of light signaling phytochromes and GA_3 act in coordination to regulate multiple aspects of arabidopsis development such as flowering and hypocotyls elongation [66].

This group of phytohormones acts throughout the life cycle of plants by influencing many physiological effects such as flower induction, stimulation of seed germination, seed pericarp growth [67], seedling emergence, stem and leaf growth, fruit growth [68], photosynthetic efficiency of plants, leaf area index, light interception, nutrient use efficiency [69], root hair abundance, promotion of root growth and inhibition of floral bud differentiation in woody angiosperms, regulation of vegetative and reproductive bud dormancy and delay of senescence in many organs of a range of plant species [70]. Under abiotic stress at a certain concentration, gibberallic acid has been shown to be beneficial for the physiology and metabolism of many plants [71].

Gibberellin production by *Azospirillum* spp. and *Bacillus* spp. has been implicated in the increased 15N uptake in inoculated wheat roots. Gibberellins act in combination with other phytohormones in highly integrated signaling pathways [72]. Gibberallic acid production by *Azotobacter, Bacillus* and *Psuedomonas* strains isolated from the rhizosphere of *Gloriosa superb* L. has been reported by Megala and Elango [73]. The actinomycetes produced three different types phytohormones indole acetic acid (IAA), gibberallic acid (GA_3) and zeatin at higher levels than those produced by symbiotic *Frankia* strain BCU110501 [74].

ACC- deaminase activity

Many microbial inoculants produce an enzyme called ACC-deaminase. This enzyme breaks down 1-amino-cyclopropane-1-carboxylic acid (ACC), which is a precursor of ethylene in plant under stress conditions. The high concentration of ethylene induces defoliation and other cellular processes that may lead to reduced crop performance [48]. Several forms of stress are relieved by ACC deaminase producers, such as effects of phytopathogenic microorganism (viruses, bacteria and fungi etc.) and resistance to stress from polyaromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, drought, extremes of temperature, high light intensity and flooding [63].

Inoculation with ACC deaminase containing bacteria induce longer roots which might be helpful in the uptake of relatively more water from deep soil under drought stress conditions, thus increasing water use efficiency of the plants under drought conditions [5]. Seed or root inoculation with ACC deaminase- producing rhizobacteria are also helpful in shoot growth, enhancement in rhizobial nodulation and N, P and K uptake as well as mycorrhizal colonization in various crops [63]. *Azotobacter,* nitrogen-fixing plant growth promoting rhizobacteria (PGPR) which survive in soil for longer period forming cyst and are known to stimulate plant growth either by facilitating the plant's uptake of certain nutrients from the environment or by production of phytohormones (auxins, gibberellins, cytokinins) [75] or by enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminasen [76]. Actinobacteria, including the genera *Micrococcus* [77] and *Gordonia* [78] also reported to produce ACC deaminase. The ability to produce ACC deaminase by some *Streptomyces* sp. from tomato and yam also reported by El-Tarabily [62] and Palaniyandi *et al* [79].

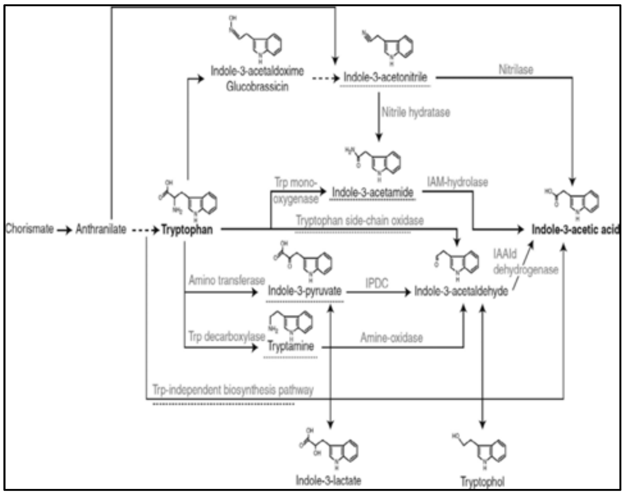

Fig 2. Different pathways to synthesize IAA in bacteria. IAAld, indole-3-acetaldehyde; IAM, indole-3-acetamide; IPDC, indole-3-pyruvate decarboxylase; Trp, tryptophan (Adapted from Spaepen *et al* [57])

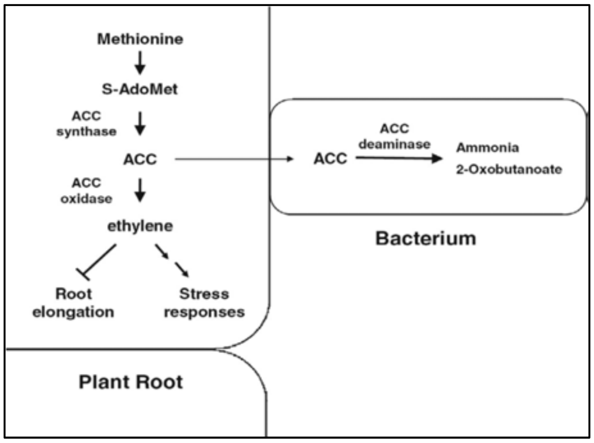

Fig 3: A possible mechanism for reduction of ethylene levels in the plant root by bacterial deaminase. S AdoMet: S-adenosyl-L-methionine; ACC: 1- aminocyclopropane-1-carboxylate (Adapted from Kang *et al* [80])

Siderophore production

Siderophores are small organic molecules produced by microorganisms under iron-limiting conditions which enhance the uptake of iron by the microorganisms. Iron is required in several metabolic processes including tricarboxylic acid cycle, electron transport chain, oxidative phosphorylation and photosynthesis [81] and plays an important role in the microbial biofilm formation [82].

Siderophore producing PGPR like *Pseudomonas* sp., *Azotobacter* [83], *Bacillus megaterium* [84] plays the vital role in stimulating plant growth and controlling several plant diseases. *Streptomyces* are most frequent producers of siderophores which produces mostly coelichelin [85] and griseobactin [86]. Verma *et al* [87] also reported that endophytic *Streptomyces* isolated from *Azadirachtaindica* produce siderophores with biocontrol potential and promote plant growth.

Phosphate solublization

Phosphorus (P) is an essential element present in all living system. In plant it is one of the least available and the least mobile mineral nutrient despite of large reservoir of P [88]. The insoluble P is present as an inorganic mineral such as apatite or as one of several organic forms including inositol phosphate (soil phytate), phosphomonesters and phosphotriesters [63]. Phosphate-solubilizing microbes can transform the insoluble phosphorus to soluble forms through production of organic acids such as gluconic acid, citric acid, succinic acid and oxalic acid [89] or through chelation, exchange reactions and polymeric substances formation [90]. The process of phosphate solubilization is also accompanied by production of plant stimulants [91], enzyme production [92], biocontrol activity [93].

Bacterial genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria [48]. Phosphorus solubilizing microorganisms have a great tendency to enhance the provision of soluble phosphate and increase the growth and development of crop plants by enhancing biological nitrogen fixation [94]. Kumar and Singh [95] established the ability of *Azotobacter* sp. to solubilizing phosphates. Wheat seed inoculation with *Azotobacter* showed increase in all the yield attributing parameters and the final yield of the crop both separately and mutually with phosphorus solubilizing bacteria [96]. Synergistic effect has been also reported between phosphorus solubilizing bacteria with nitrogen fixers including *Azospirillum* and *Azotobacter.* Kumar *et al* [97] also disclosed that a rock phosphate solubilizing and phytohormone-producing *Azotobacter chroococcum* resulted in 11.4% increase in wheat yield over the control. *Streptomyces griseus* and *Micromonospora aurantiaca* related strains also, known for their rock phosphate solubilization abilities [98].

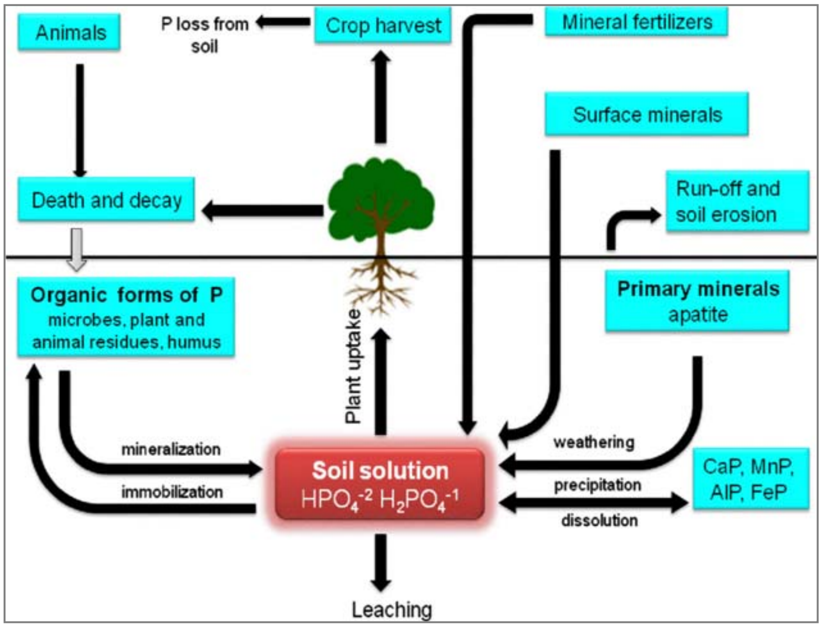

Fig 4. Phosphorus mobility in soil adapted from Ahemad and Kibret [24]

Ammonia production

Nitrogen (N) is the most essential nutrient for growth and productivity of plant. About 78% N_2 present in atmosphere but it is unavailable to the growing plants. Dinitrogen becomes available through the biological nitrogen fixation process that is confined to prokaryotic cells, including some eubacteria, cyanobacteria, and actinomycetes [99, 100]. These include symbiotic nitrogen fixing (N2-fixing) forms, viz. *Rhizobium,* the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees, and non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena, Nostoc*), *Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus and Azocarus* etc. [48]

Azotobacter is generally regarded as a free-living aerobic nitrogen-fixer. *Azotobacter paspali,* was first discovered from the rhizosphere of *Paspalum notatum*. *Azotobacter* strains affect seed germination, seedling growth in a plant, and have been used as effective inoculums to enhance plant growth and pest control [101] as it carries many beneficial characteristics such as carbon utilization, IAA production, ammonia excretion and nitrogen fixation [102]. According to Kumar *et al* [97] wheat which has been inoculated with free nitrogen fixators grows more evenly and has a higher yield. Oztrurk *et al* [103] also reported that biofertilization activates microbiological processes in soil and part of nitrogen fertilizers in wheat production can be replaced by microbiological fertilizers.

According to Revillas *et al* [104] rhizospheric bacteria such as *Azotobacter*, *Arthrobacter* and *Streptomyces* have strong beneficial effects on plant growth and integrity by nutrient dissolution, nitrogen fixation and through the production of plant hormones and vitamins. Wani *et al* [105] reported the importance of *Azotobacter*, in nitrogen fixation in rice crops and it has been use as a biofertilizer for wheat, barley, oat, rice, sunflowers, maize, line, beetroot, tobacco, tea, coffee and coconuts. It has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation [106].

Need for the adaptability of microbial inoculants over wide range of pH, temperature and salinity

The critical factor that may be affecting the activity of microbial inoculants in soil is pH, environmental temperature and salinity of the soil.

During the past 100 years, India has experienced significant increase of 0.6 °C surface air temperature which is considered higher than that for the previous century. In Punjab state, during the last 40 years, the mean minimum temperature and humidity level had gone up by 1 °C and 8-10% respectively thus revealing change in climatic value [107].

The Indian soils has been characterized by pH values >7 and low to high salinity ($EC_{1:5}$ from 0.09 to 6.9 dS m^{-1}) and ranged from being non-sodic to saline-sodic (SAR from 0.19 to 20.3) [108].

Soil acidity is the one of the most yield limiting factor for crop production. Most of the south eastern soils are inherently more acidic in nature than soil of the Midwest and far West. Plant available forms of nitrogen, sulfur and phosphorus are reduced in acidic soils and symbiotic nitrogen fixation by leguminous crops because nutrient transformation and nitrogen fixation are restricted in acidic soils, which is due to reduced activity of the microorganisms that are responsible for carrying these processes. The availability of nutrients to plants is altered by soil pH.

Tejera *et al* [49] assessed growth rates of *Azotobacter sp*. at different initial pH values (4-9) and showed that a lower number of isolates grew on N-free media at pH value as high as 8-7. Diversity is highest in neutral soils and minimum in acidic soils [109, 110]. At low temperature condition, growth rates of *Azotobacter* had reported to declined by the decrease in membrane fluidity and enzyme activities [111].

Similarly, more than 800 million hectares of lands throughout the world are salt-affected and equating to more than 65 of the world's total land area [112]. In India approximately, 25 Mha of soils are affected by soil acidity which is 30% of the current area under cultivation [113]. And about, 6.7 Mha land is affected by salt stresses including 3 Mha by slanity and 3.7 Mha by alkalinity, distributed in 15 of the 29 states.

Vessey [114] stated that any microbial inocoulant including PGPR to be used as biofertilizer is likely to be more successful under field conditions with additional characteristics like higher salt or high temperature tolerance. Biofertilizer could play an important role in stress management in the edaphic stress prone areas. Thus adaptability of microbial inoculants over wide range of pH, temperature and salt concentration is crucial for their application under different agroclimatic conditions.

Microbial inoculants: A green biotechnological approach

The biological commence for convalescent crop production are gaining strong dignity among agronomist and environmentalists following integrated plant nutrient management system. There is ongoing rigorous research worldwide to explore a wide range of rhizobacteria possessing peculiar characteristics like ammonia production, nitrogenase activity [63], phosphate soliblization [115], 1- amino-cyclopropane-1-carboxylate, hydrogen cyanate (HCN), siderophore [116], salinity tolerance [117], biological control of phytopathogens and insects [118] alongwith the normal plant growth promoting properties such as phytohormone, pesticide degradation/ tolerance [119] and heavy metal detoxifying potentials [120, 121]. Diverse group of symbiotic (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*) and non-symbiotic (*Psuedomonas*, *Bacillus*, *Klebsiella*, *Azotobacter*, *Azospirilum*, *Azomonas*) rhizobacteria are now being use worldwide as bio-inoculants to promote plant growth and development under various type of biotic and abiotic stresses.

Formulation that contains one or more beneficial bacterial strains or species in an economical and easy-to-use carrier method called bacterial inoculant. Inoculation is the mean of transport of living bacteria from the factory and introduces them on to plant so they produce the felicitous effects on plant growth. Peat is the most successively used carrier inoculant because of high surface area and high water holding capacity. In many countries, peat is not available especially in tropics and will be depleted in the future in many areas. Peat based inoculants carrier requires a perceptible amount of processing, such as milling, mining, neutralizing and drying before used by commercial production system. A costly investment in equipments is required for processing of peat and it is usually not feasible for small production operation. Peat based carriers are not easy to use with sophisticated planting equipment [122].

To the problems associated with the processing of solid carriers, liquid inoculants formulations are the solution. In the liquid inoculant formulations, various broth cultures are amended with agents that promote cell survival in the package and after application to the seed.

Liquid inoculants can be easily adapted to advanced seeding equipment, it can be sprayed onto the seed as it passes through the seed auger and dries before it travels into the seed bin on the planter. Addition of sucrose, glycerol, gum Arabic, poly vinyl pyrrolidone (PVP) improves survival of microorganisms in liquid inoculants [123]. These additives are used to improve the quality of inoculant through inactivation of the toxins, a better adhesion to seed, through enhancement of the strain survival during storage and after exposure to extreme environmental conditions (desiccation, high temperature) and stabilization of the product after inoculation [124].

Liquid formulation has several advantages such as high cell count, no contamination, longer shelf life, greater protection against environmental stress and increased field efficacy [124, 125]. Chang *et al* [126] proposed the use of polyethylene glycol (PEG)-g-chitosan for cell adhesion applications. Temprano *et al* [127] also reported the use of various polymer like Poly vinyl pyrrolidone (PVP), Poly ethylene glycol (PEG) and gum Arabic because of their sticky consistency which may enhance cell adherence to seed and their viscous nature slow down the drying process of the inoculants after application to seed.

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