Review Article
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Role of *Azotobacter* in soil fertility and sustainability–a review

Abstract

In the preset context, the best alternative of chemical fertilizer is necessary because of its adverse effects on the soil health. There are several alternatives available to enhance the soil fertility one of them is Azotobacter. It is a free–living N2– fixer diazotroph that has several beneficial effects on the crop growth and yield. It helps in synthesis of growth regulating substances like auxins, cytokinin and Giberellic Acid (GA). In addition, it stimulates rhizospheric microbes, protects the plants from phyto–pathogens, improves nutrient uptake and ultimately boost up biological nitrogen fixation. The abundance of these bacteria in soil is related to many factors, mostly soil pH and fertility.

Keywords: *azotobacter*, biofertilizers, biological nitrogen fixation, soil sustainability

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Introduction

Azotobacter spp. are Gram negative, free–living, aerobic soil dwelling,[1] oval or spherical bacteria that form thick–walled cysts (means of asexual reproduction under favorable condition).[2] There are around six species in the genus *Azotobacter*[3] some of which are motile by means of peritrichous flagella, others are not. They are typically polymorphic and their size ranges from 2–10µm long and 1–2µm wide.[2] The *Azotobacter* genus was discovered in 1901 by Dutch microbiologist and botanist Beijerinck et al.[4] (founder of environmental microbiology). *A chroococcum* is the first aerobic free–living nitrogen fixer.[4]

These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants. *Azotobacter spp*. is sensitive to acidic pH, high salts, and temperature.[5] *Azotobacter* has beneficial effects on crop growth and yield through, biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phyopathogenic inhibitors.[6–7] Modification of nutrient uptake and ultimately boosting biological nitrogen fixation.[8]

Besides being quite expensive and making high cost of production, chemical fertilizers have adverse effect on soil health and microbial population. In such situation, biofertilizer can be the best alternative for enhancing soil fertility. Being economic and environmental friendly, biofertilizers can be used in crop production for better yield.[9] Similarly, microbial products are considered safer, self–replicating, target specific, which is regarded as major component of integrated nutrient management from soil sustainability perspective.

Role of Azotobacter in soil fertility

***Azotobacter* in soil:** The presence of *Azotobacter* sp. in soils has beneficial effects on plants, but the abundance of these bacteria is related to many factors, soil physico–chemical (e.g. organic matter, pH, temperature, soil moisture) and microbiological properties.[10] Its abundance varies as per the depth of the soil profile.[11–15] *Azotobacter*ia are much more abundant in the rhizosphere of plants than in the surrounding soil and that this abundance depends on the crop species.[16,17]

Nitrogen fixation: Nitrogen is the component of protein and nucleic acids and chlorophyll. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crop.

The atmosphere comprises of ~78% nitrogen as an inert, in unavailable form. Above every hectare of ground there are ~80000 tones of this unavailable nitrogen. In order to be converted to available form it needs to be fixed through either the industrial process or through Biological Nitrogen Fixation (BNF). Without these nitrogen–fixers, life on this planet may be difficult.

Nitrogen (N) deficiency is frequently a major limiting factor for crops production.[18] Nitrogen is an essential plant nutrient, widely applied as N–fertilizer to improve yield of agriculturally important crops. An interesting alternative to avoid or reduce the use of N–fertilizers could be the exploitation of Plant Growth–Promoting Bacteria (PGPB) capable of enhancing growth and yield of many plant species, several of agronomic and ecological significance.

*Azotobacter*spp. are non–symbiotic heterotrophic bacteria capable of fixing an average 20kg N/ha/per year.[10] Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism.[19]

Seed inoculationAzotobacterand nutrient uptake

Seed Inoculated with *Azotobacter* helps in uptake of N, P along with micronutrients like Fe and Zn, in wheat, these strains can potentially be used to improve wheat nutrition.[20] Seed inoculation of *Azotobacter* profoundly contribute to increase yield by supplying nitrogen to the crops. Inoculation of seeds with *Azotobacter chroococcum*increased carbohydrate and protein content of two corn varieties (Inra210 and Inra260) in greenhouse experiment.[21] There is increment in Maize biomass with the application of manure and *Azotobacter*.[22]

In nitrogen–deficient sand, seed inoculation increased plant length, dry weight, and nitrogen content in addition to a significant increase in soil nitrogen.[23] It was found that *A. chroococcum*at concentration of 108cfu ml^{-1} increased seed germination of Cucumber.[2] Seeds of wheat (*Triticum aestivum*) were inoculated with 11 bacterial strains of *A.chroococcum*, Research result showed that all *A. chroococcum*s trains had positive effect on the yield and N concentrations of wheat.[24]

Role of Azotobacter in growth substances production and promotion

Besides, nitrogen fixation, *Azotobacter* produces, Thiomin, Riboflavin, Nicotin, Indol Acetic Acid and Giberalin. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent. Brakel et al.,[25] showed that *Azotobacter* produced Indol–3–Acetic Acid (IAA) when tryptophan was added to the medium. Hennequin et al,[26] found only small amounts of IAA in old cultures of *Azotobacter* to which no tryptophan was added.

Bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and GA–like substances, and these growth materials are the primary substance controlling the enhanced growth of tomato.[27] These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants. Eklund et al.,[28] demonstrated that the presence of *Azotobacter chroococcum* in the rhizosphere of tomato and cucumber is correlated with increased germination and growth of seedlings. Puertas et al.,[29] report that dry weight of tomato plants inoculated with *Azotobacter chroococcum* and grown in phosphate–deficient soil was significantly greater than that of non inoculated plants. Phytohormones (auxin, cytokinin, gibberellin) can stimulate root development.

High Gibberillic acid production was detected in *Azotobacter*(71.42 %) isolates. Higher phosphate solubilization was detected in the isolates of *Azotobacter* (74.28 %) followed by *Pseudomonas* (63.00 %).[30] Gibberellins applied in small quantities to the soil or rosettes on the leaves and shafts of certain plant produces an increase in height. In grains such as wheat and corn, they also cause an increase in length of the leaves. In some cases they also increase the fresh weight and dry weight. However it doesn't produce any effect on the growth of the roots.[31]

Brown et al.,[32] have found that after treating tomato seeds or seedling roots with small amounts (0.5–0–01pg.) of commercially produced gibberellins GA 3**,** the plants responded in the same way as after treatments with 14–day cultures of *Azotobacter chroococcum*strain A 6**.** *Azotobacter chroococcum*strains isolated from the sugar beet rhizosphere were also shown to produce gibberelins; the growth of pea hypocotyl was equivalent to a GA3 concentration of 0.003–0.1μg/cm^3 culture.[33] Similarly, Cytokinins are related to nucleic acids with precursor substances that act to stimulate cell division in vegetative growth areas.

These responses suggest that *Azotobacter* probably influences the development of plants by producing growth–regulating substances. Therefore, *Azotobacter* spp. is often regarded as a member of "Plant Growth Promoting Rhizobacteria (PGPR)".

It was also concluded that *Azotobacter* inoculants have a significant promoting effect on growth parameters like root, shoot length and dry mass of bamboo and maize seedlings in vitroand in pot experiments.[2] The dual inoculation of *A. chroococcum*and *P. indica* had beneficiary response on shoot length, root length, fresh shoot and root weight, dry shoot and root weight, and panicle number that affect growth of rice plant.[34]

*Adathoda vasica*plants inoculated with *A. chroococcum* revealed significantly increased root nitrogen content compared to the control plants.[35] The *Azotobacters* which were inoculated with *Rhizophora* seedlings, increased significantly the average root biomass up to by 98.2%, the root length by 48.45%, the leaf area by 277.86%, the shoot biomass by 29.49% as compared to controls and they also increased the levels of total chlorophylls and carotenoids up to by 151.0% and 158.73%, respectively.[36] The *Azotobacter* inoculated crop show better growth rate. During rosette stage of canola the Crop Growth Rate (CGR) found highest i.e. 10–12 % increment.[37]

Dry matter accumulation

There is increment in dry matter accumulation in *Azotobacter* inoculated plants; it stimulates development of foliage, roots, branching, flowering and fruiting which is triggered by fixed nitrogen and plant growth regulator like substance produced. It also increases plant tolerance to lack of water under adverse condition.[38] Similar result put forwarded by Sandeep et al.[39] which revealed that there is better growth response of *Azotobacter* inoculated plants as compared to non–inoculated control plants. Better crop growth response ultimately results in better dry matter accumulation.

Leaf Area Index (LAI)

*Azotobacter*in balanced nutrient condition results in 3.5 % increment in LAI at rosette stage of canola crop and additional application of *Azotobacter*shot up the yield by 21.17 % over the control (chemical fertilizers).[37] The rate of increase in the leaf area determines the photosynthetic capacity of plant, which leads to better assimilation of produce and towards yield. Using *Azotobacter* spp. potato yield has been increased by 33.3% and 38.3%.[38] Similarly 20% yield increment is recorded in *Azotobacter* inoculated plants[40,41] reported that, number of branches, pod per plant and 1000 grain weight also increased with *Azotobacter* application.

Biochemical effects

Several strains of *Azotobacter* are capable of producing amino acids when grown in culture media amended with different carbon and nitrogen sources.[42] Substance like amino acid produced by these rhizobacteria are involved in many processes that explain plant–grown promotion. Biochemical analysis of chlorophyll, nitrogen, phosphorous, potassium and protein content was higher in *Azotobacter* inoculated plants as compared to non–inoculated control plants.[43]

Anti–pathogenic response

Azotobacter spp. are capable to produce siderophore, they bind to the available form of iron Fe+3 in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant health; similarly Hydro Cyanine (HCN) production was higher in traits of *Azotobacter* (77.00 %).[30] *Azotobacter* secretes an antibiotic with a structure similar to anisomycin, which is a documented fungicidal antibiotic. *Azotobacter*, in sufficient numbers, will out–compete pathogens for food. Some of the pathogens that have been controlled by *Azotobacter*in the soil and on the leaf include: *Alternaria, Fusarium, Collectotrichum, Rhyzoctonia, Microfomina, Diplodia, Batryiodiplodia, Cephalosporium, Curvularia, Helminthosporium*and *Aspergillus.*

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Effects of chemical fertilizer in Azotobacter

Combined application of bio–fertilizer with 50% of chemical fertilizers (N and P) has significant effect in plant growth, plant height, number of branches, fresh and dry weight of safflower in comparison with chemical fertilizers alone. Similarly, application of *Azotobacter* biophosphate and organic fertilizers, with half dose of chemical fertilizers increase the economic yield of safflower.[44] Efficiency of *Azotobacter* found decreased with increased N level.[45] The best combination was recorded with NH4Cl at 0.1g/L whereas, action of copper in *Azotobacter* found toxic even in very low concentration.[46] The population of *Azotobacter* may suffer due to high amount of nitrates and the acidic environment created because of chemical fertilizer.

Effect of pesticides in Azotobacter

Balajee et al.,[47] reported that, the effect of herbicide 2, 4–D and its products; p–chlorophenoxy–acetic acid and p–chlorophenol were utilized by *A. croococcum* as carbon source, which ultimately stimulate nitrogenase enzyme. Similar result found by Kanungo et al.,[48] which revealed insecticide carbofuran also stimulates nitrogenase enzyme activity. Martinez et al.,[49] found that, herbicide simazine have no effect on *A. chroococcum* growth either on standard medium or on dialysed soil and sterilized soil medium. The presence of 50–300mg of simazine per ml of culture or in one gram of soil did have a stimulating effect on *Azotobacter*. When *Azotobacter* is grown in presence of simazine, the cells have a higher ATP content than the control. Whereas organophosphorous insecticides profenofos and chloropyrifos had adverse effect on the number of aerobic nitrogen fixers and decreased nitrogen fixation.[50]

Azotobacter in nutrient cycling

*Azotobacter*makes availability of certain nutrients like Carbon, Nitrogen, Phosphorus and Sulphur through accelerating the mineralization of organic residues in soil and avoid uptake of heavy metals.[51] *Azotobacter* can be an important alternative of chemical fertilizer because it provides nitrogen in the form of ammonia, nitrate and amino acids without situation of over dosage, which might be one of the possible alternatives of inorganic nitrogen source (eg. Urea). It also helps to sustain the plant growth and yield even in case of low phosphate content soil, as well as helps in uptake of macro and certain micro nutrients which facilitates better utilization of plant root exudates itself.

Conclusion

Azotobacter spp. are free living, non–symbiotic, heterotrophic bacteria capable of fixing an average of 20kg N/haper year. These bacteria are regarded as Plant Growth Promoting Rhizobacteria (PGPR) which synthesize growth substance that enhances plant growth and development and inhibit phytopathogenic growth by secreting inhibitors. It also helps in nutrient uptake and produces some biochemical substances such as protein, amino acids etc. *Azotobacter* improves seed germination and has beneficiary response on Crop Growth Rate (CGR). It helps to increase nutrient availability and to restore soil fertility for better crop response. It is an important component of integrated nutrient management system due to its significant role in soil sustainability. More research is necessary in future to explore the potentiality of *Azotobacter* in soil fertility.

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Conflict of interest

The author declares no conflict of interest.

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