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Use of *Azospirillum* and *Azobacter* bacteria as biofertilizers in cereal crops: A review

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Abstract

Biofertilizer is a wide term which includes a diverse category of bioinoculants such as nitrogen fixers, phosphate solubilizers, phosphate mobilizers and plant growth promoting rhizobacteria. Numerous bacterial species have found as PGPR mainly Azotobactor, Azosprillium, Bacillus, Pseudomonas etc. The application of these bacterial species as biofertilizers could be the alternate source of synthetic fertilizers because these bacterial species have great potential to fix atmospheric nitrogen as well as to solubilize the phosphorus in the soil. *Azotobacter* and *Azospirillum* genera are free-living bacteria and fix atmospheric nitrogen in cereal crops without any symbiosis. They fix 20-40 kg ha^{-1} nitrogen per year. *Azotobacter* sp. also has ability to produce antifungal compounds against many plant pathogens. Thus, biofertilizers containing beneficial organisms are cost effective, pollution free and a perennially renewable source of plant nutrients, making them ideal partners and essential supplements to chemical fertilizers.

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Introduction

Biofertilizers are a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The various microorganisms used as nitrogen-supplying biofertilizers are rhizobium, actinorhizae, azotobactor, and azospirillum. They are used for leguminous as well as non-leguminous crops. Free-living nitrogen-fixing bacteria were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Fayez et al, 1985). The genera *Azospirillum* and *Azobacter* show generalized occurrence in economically important cultures such as corn, wheat, rice and sorghum, like this being with frequency, in experiments seeking the agronomic utilization as biofertilizers (Döbereiner, 1997; Reinhold and Hurek, 1988; Sundaram et al, 1988). Motsara et al (1995) reported that inoculation of *Azospiril1um* and *Azotobacter* exerted beneficial effect on yield with varying physiological activities, including synthesis of plant growth promoting substances. Furthermore, inoculation of PGPR can increase plant uptake of several other nutrients such as Ca, K, Fe, Cu, Mn and Zn. This uptake usually occurs during acidification of the soil rhizosphere via organic acid production or via stimulation of proton pump ATP ase (Mantelin and Touraine, 2004).

Advantages of biofertilizers:

1- Reduce the use of chemical fertilizers.
2- Reduction of environmental pollution.
3- Increase the validity of nutrients and easily absorbed.
4- Excretion of doping substances for growth.
5- Improve the physical, chemical and biological properties of the soil.
6- Excretion of some antibiotics that is resistant to some plant diseases.
7- Biofertilizers are not costly and even poor farmers can make use them.
8- Microorganisms convert complex organic material in simple compound, so that plant easily taken up.
9- Enhances root proliferation due to release of growth promoting hormones.
10- Environmentally friendly, cleanse the plant from precipitated chemical fertilizers.

1- Biofertilizers are sensitive to temperature and humidity changes, hence difficult to store.
2- Their effects are is slower than chemical fertilizer.
3- In rural and remote areas, often it’s hard to find a retailer selling biofertilzers.
4- Biofertilizers complement other fertilizers, but they cannot totally replace them.
5- Shortages of particular strains of microorganisms or of the best growing medium reduce the availability of some biofertilizers.
6- Other chemicals should not be mixed with the biofertilizers.

The role of *Azospirillum* in cereal crop production:

Azospirillum is a Gram negative motile bacteria belonging to the order *Rhodospirillales*, associated with roots of monocots, including important crops, such as wheat, corn and rice. *Azospirillum* is the primary commercial phytostimulator inoculant for cereals worldwide. *Azospirillum* can establish an associative symbiosis with cereals but unlike mutualistic symbiosis, the association is not accompanied by the formation of new organs. *Azospirillum* benefits the plant directly, via associative nitrogen fixation, synthesis of phytohormones (notably indole-3-acetic acid, IAA), and modulation of plant hormonal balance by deamination of the ethylene precursor. It is an associative type of microorganism capable of colonizing the root surface of the plants. By establishing a symbiotic association ship, it helps the plants in getting the nutrient nitrogen from the atmosphere.

Inoculation of wheat with Azospirillum sp. increased root dry weight and 1000-kernel weight (Jagnow 1990), raised number of spikes per plant, number of grains per spike and grain and straw yield (Darmwall and Gaur 1988, Ozturk et al 2003), N-uptake and N-yield (Bhattarai and Hess 1993) in wheat. Inoculation with Azospirillum produced significantly higher grain yield by 29% and the grains contain more N (22.8%), P (59.5%) and K (34%) compared to the control plants (Askary, et al 2009).

A strong increase in total plant and grain dry weight was obtained when maize plants were inoculated with *Burkholderia cepacia*, *Azospirillum brasilense* and *H. seropedicae* in individual experiments, in comparison to plants grown in soils without nitrogen (Riggs et al. 2001). Salomone and Dobereiner (1996) observed significant increase in grain yield of maize on inoculation with Azospirillum spp. Swędrzyńska, and Sawicka (2000) found that inoculation of maize crops with an active strain of Azospirillum brasilense has a beneficial effect on maize vigor and yield.

Increase in different plant parameters such as height, tiller number, dry matter yield and N uptake of rice plants that inoculated with Azospirillum lipoferum (Nayak, 1986 and Murty, 1988). Inoculation of Azospirillum sp. to wetland rice under acidic condition improved shoot growth, straw yield and N uptake (Govindan and Bagyaraj, 1995). Azospirillum inoculation could significantly increase the growth in terms of height; number of leaf/plant; length and breadth of leaf; and fresh and dry weight/plant of rice plant (Hossain, et al 2015). Inoculation of rice plants with Azospirillum has been found to cause significant increases in growth and yield which is equivalent to that is attainable by application of 15-20 kg N/ha (Rodrigues et al., 2008).

Inoculation by Azospirillum increased total dry matter and seed yield in sorghum up to 10-30 percentage compared with control (Kapulnic et al., 1981). Effect of different N fertilizer levels and biofertilizers on forage sorghum indicated that using of 75 kg/ha N (urea), 25 kg/ha N (castor residuum) and inoculation by Azospirillum increased the raw protein and quality of forage (Yadav et al., 2007).

Seed inoculation with A. brasilense increased the mean grain yield of pearl millet under different agroclimatic conditions in India. Also, the root biomass increased with Azospirillum spp. inoculation at 10 and 20 kg N/ha than their corresponding uninoculated controls (Tilak and Subba Rao, 1987). Abdullahi, et al (2014) found that bio-fertilizer (Arbuscular mycorrhizal fungi and Azospirillum brasilense) + poultry manure recorded highest plant performance viz; plant height, number of tillers/plant, shoots and root dry biomass of pearl millet. Results also showed that bio-fertilizer tended to reduce by half the application rates of organic manure.

The role of *Azotobacter* in cereal crop production:

These are free living bacteria which grow well on a nitrogen free medium. 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*.,are sensitive to acidic pH, high salts, and temperature above 35^0C. There are four important species of *Azotobacter* viz. *A.Chroococcum*, *A.agilis*, *A.paspali* and *A.vinelandii* of which *A.chroococcum* is most commonly found in Egyptian soils.

Besides N_2 fixation, *Azotobacter* synthesizes and secretes considerable amounts of biologically active substances like B vitamins, nicotinic acid, pantothenic acid, biotin, heteroxins, gibberellins etc. which enhance root growth of plants (Rao, 1986). Another important characteristic of *Azotobacter* association with crop improvement is secretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta, 1986).

Improvement in crop production due to *Azotobacter* inoculation has been reported in a number of crops. *Azotobacter* increases the yield of all the agriculture crop plants about 10-12 % (Jaga and Singh 2010). *Azotobacter* can also able to enhance the growth and grain yield in wheat crop (Kader, et al 2002; Abd El-Lattief, 2014). Azotobacter plays a very important role in the growth of plants especially it improves the yield of wheat. The yield of wheat increases when it was inoculated with yeast + Azotobacter with 20 m^{-3} fad (Ahmed et al, 2011). Yousefi and Barzegar (2014) indicated that the combined application of Azotobacter and Pseudomonas increased grain yield, harvest index, biological yield and protein content of wheat by 34.3, 7.7, 12.5 and 13.6%, respectively compared to the controls. Azotobacter and Pseudomonas inoculation plus fertilization reduced chemical fertilizers application (25-50 %) in the field.

Inoculation of maize plants with Azotobacter increased 15 to 35% grain yield over non inoculated treatments (Baral and Adhikari, 2013). Grain yield of maize increased with inoculation by Azotobacter (Gholami et al, 2009; Hajnal-Jafari et al, 2012; Naseri et al, 2013).

Azotobacter act as one of the important biofertilizer for rice and other cereals, it can apply by seed dipping and seedling root dipping methods (Kannaiyan, et al 1980; Kannaiyan, 1999; Singh, et al 1999; Rüttimann, et al 2003).

Reddy et al (1977) found that seed inoculation with Azotobacter increased grain yields of sorghum by 19.5% in 1975 and 15.2% in 1976. Jat et al (2013) found significant enhancement in grain (26.8 and 28.5%) and stover (11.9 and 13.0%) yields of sorghum when application Azotobacter + PSB over no inoculation.

Inoculation of seed with Azotobacter resulted higher plant height at time of harvest, yield attributing characters and yield of pearl millet (Patel et al, 2014).

The combined inoculation of *Azotobacter* and *Azospirillium* in cereal crop production:

Rai and Caur (1998) studied *Azotobacter* and *Azospirillum* and double-inoculation and alone inoculation effects on wheat growth and yield. Double-inoculation of *Azotobacter* and *Azospirillum* had positive effects on grain yield, biological yield and harvest index in various wheat genotypes.

Kandil et al. (2011) studied the effects of inoculation with Azotobacter sp. and Azospirillum sp. on wheat and observed that inoculated wheat plants gave higher plant height, spike per unit of area, grains per spike, grain weight, biological yield, grain yield and straw yield compared to non-inoculated cultivars.

Among the different inoculations, combined application of Azotobacter spp. and Azospirillum spp. was found most efficient and resulted in maximum values of plant growth parameter, yield attributing characteristics, grain yield (1.23 Mg/ha), soil microbial biomass carbon (SMBC) and dehydrogenase activities at all the growth stages of common buckwheat (Singh, et al 2015)

According to Das and Saha (2007), combined inoculation of Azotobacter, Azospirillium along with diazotrophs increased grain and straw yield of rice by 4.5 and 8.5 kg ha^{-1}, respectively.

Conclusion:

Biofertilizers as a better supplement can improve the growth and yield of cereal crops. Nitrogen fixing biofertilizers mainly *Azospirillum* and *Azotobacter* can able to fix 20-40 kg N/ha and produce growth promoting substances like IAA. Inoculation with asymbiotic nitrogen fixers like *Azospirillum* or

Azotobacter may improve plant growth and yield due to supplementing the growing plants with fixed nitrogen and growth-promoting substances. Hence, it is imperative to popularize the use of biofertilizers, which is a low-cost input technology to reduce the dependence on inorganic fertilizers and contribute to pollution-free atmosphere, which is the need of the day.

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