

Mechanisms of Azospirillum in Plant Growth Promotion

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Abstract: *Azospirillum* is one of the successful inoculants for di-nitrogen fixation and plant growth promotion in non-legume crops. It is micro-aerophilic microorganisms found mainly with cereals and grasses. Apart from dinitrogen fixation, it confers many other benefits to crop plants like improve seed germination, enhance seedling growth, enhancing proton flux, phosphorus solubilization, sequestration of iron, production of phytohormones, increase photosynthetic pigments, helping other plant growth promoters, enhance dry matter partitioning, restoration of vegetation in harsh environment, enhance seed quality, alleviate biotic and abiotic stresses, etc. It can be used as microbial inoculants of interest for overall success of plant in the field. I also enhance the adaptability of other bio-inoculants by providing wider benefits. In this mini-review, we are discussing such mechanisms which *Azospirillum* confers to plants.

Keywords: *Azospirillum*, plant growth promotion, nitrogen fixation, stress tolerance

INTRODUCTION

Microbial inoculants provide colossal benefits to crops starting from seed germination to prevention of post-harvest losses [1, 2]. Beneficial microbes confer sustainable plant performance by nitrogen fixation, P-solubilization, K-solubilization, Zn solubilization, Fe-sequestration, phytohormones production, induced systemic resistance, induced systemic

tolerance, etc. [2-12]. *Azospirillum* is one of the most successful microbial inoculants found in cereals and grasses. *Azospirillum* is non-symbiotic plant growth promoter which attaches as aggregates on root surface embedded in mucigel layer or penetrating into root intercellular spaces, preferably in the elongation and root hair zones [13]. There are various mechanisms, either major or minor, additively makes overall improvement in plant growth.

A plenty of reports are available on growth enhancements of crops like cereals [14, 1] and vegetables [15]. In maize crop, *Azospirillum* inoculation found enhancing the crop yield in the range similar to 60 kg urea N/ha [16]. Millets and guinea grass have shown a saving in 39–42 kg N with *Azospirillum lipoferum* inoculation [17]. An increase of 10 to 30% was reported due to *Azospirillum* inoculation in field grown plants [18, 19]. A few reports indicated extremely higher values, 50-270% over non-inoculated controls. In cotton, about 70-75% of all pot experiments showed increase in yield [15].

There many mechanisms by which *Azospirillum* enhances plant growth like N₂ fixation,

hormonal effects, nitrate reductase activity, etc. A detailed description of plant growth promotion activities are as follows-

Nutrient Acquisitions

N₂ fixation

Data for effects of inoculation on yield 20 years indicated that 60–70% of field experiments were successful [20] and an increase of 5-30 per cent was recorded. Logically, credit can't be given to nitrogen fixation alone; as the bacterial nitrogen fixation contributes to plant growth is usually range from 5-18% of total plant increase [21]. There are many other mechanisms involve in effectiveness of *Azospirillum*. Increase nitrite production also increase formation of lateral roots. Increase nitrate accumulation-Increase biomass and nitrate content [22].

Increases the proton efflux of the roots

Azospirillum brasilense secretes some bacterial signals [23] which cause changes in phospho-lipid of membrane and efflux of protons [23]. This change in membrane activity reduces pH [24] of the soil solution which results in solubilisation of ions like PO₄²⁻. It also increases the availability. Increased permeability of

roots enhances the uptake of minerals by plants and improves growth [25].

Production of gluconic acid and phosphorus solubilisation

There are two mechanisms for phosphate solubilisation which are production of organic acids or enzyme production. It is rather interesting to note the production of gluconic acid by the *Azospirillum* spp. Usually *Azospirillum* utilizes the organic acid, doesn't produce it [26] but in the presence of glucose as a carbon source, *Azospirillum* does produce gluconic acid. Again, *Azospirillum brasilense* can't grow in the glucose as a carbon source. Hence, gluconic acid is produced in the media only when the glucose is amended with fructose. This gluconic acid solubilises the phosphorus in soil [27] and thus can help plant in improving phosphorus nutrition.

A. brasilense and *A. lipoferum* found giving clear zones of P solubilisation in media containing calcium phosphate. It shows *in-vitro* gluconic acid production and phosphate solubilization by *A. brasilense* and *A. lipoferum*. *Azospirillum* is a microbe of immense importance and P-solubilization is an added benefit from it.

Iron Sequestration

Iron is one of the most critical nutrient elements required by all life forms. It is available usually in very small quantity in soil solution. Uptake of iron by *Azospirillum* is attributed to siderophore production. *Azospirillum lipoferum* produces derivatives of Dihydroxybenzoic acid (DHBA) i.e. 2,3-DHBA and 3,5-DHBA leucine and lysine conjugates [28]. Gluconic acid is one of the favoured carbon sources of *A. lipoferum* for producing siderophores [29]. These siderophores sequester the iron for plants and starve the pathogens off it.

Plant Growth Promotion

Improves the seed germination and germination parameters

Puente and Bashan [30] inoculated wild *Cardon cactus* seeds with *Azospirillum brasilense* strains Cd and Sp-245 for improving seed germination and other parameters. *A. brasilense* Sp245 showed significantly higher seedling germination of *Cardon cactus* but the other strain *A. brasilense* Cd found decreasing the germination. At the same time, both bacterial strains improved several parameters related to seedling survivability i.e. height, diameter, volume and the volume/surface ratio and shortened the aging process of the seedling spines. Both strains found surviving in the cactus rhizosphere for about 300 days after seed inoculation. This study evident that *A. brasilense* can be used in wide range of crops. It can be used in nurseries to enhance seedling growth.

Plant growth hormones

Production of phytohormones is one of plant growth promoting activity of *Azospirillum*. Perrig *et al.* [31] evaluated phytohormone biosynthesis and other parameters in two strains (Cd and Az39) of *Azospirillum brasilense* used for inoculant formulation in Argentina during the last 20 years. GC-MS and HPLC were used for detection of Indole 3-acetic acid (IAA), gibberellic acid (GA3), and abscisic acid (ABA) ethylene, and zeatin (Z). Both strains found producing phytohormones (IAA, Z, GA3, ABA and ethylene) and growth regulators (putrescine, spermine, spermidine, and cadaverine). These growth promoters may be the cause of significant yield increase in the plants. Plant hormones also reported to have effects on dinitrogen fixation [32].

The major hormone produced is indole-3-acetic acid (IAA) [33, 34]. Other hormones detected were indole-3-butyric acid (IBA) [35], indole-3-ethanol, indole-3-methanol [36], unidentified indole compounds [33] gibberellins [37] abscisic acid (ABA) [38] and cytokinins [39, 31]. There are reports of higher amounts of IAA and IBA production in roots of inoculated maize than in uninoculated control [35].

- Changes in root length [38]
- Produces more root hairs [40]
- Branching of root hairs [41]
- Produces more lateral roots [34]
- Enhances the rate of cell division and differentiation in meristematic tissues [35].

Studies conducted with mutants shows that IAA overproducing *Azospirillum* mutant strongly affected plant root morphology [41] but the mutant that failed to produce IAA doesn't affect root morphology [42]. If hormone-defective mutant of wheat is inoculation with *Azospirillum* improved the hormonal balance in plant [43].

Participates in dry matter partitioning in grasses

In order to maintain functional equilibrium in the plants, all the parts i.e. root, stem and leaves should be in right proportion. This partitioning of dry matter for a balanced R.S ratio is a combine result of numerous large and small processes at different levels. *Azospirillum* too influence the dry matter partitioning of plants by several mechanisms e.g. N₂-fixation, P-solubilization, phytohormone production, etc. [44].

Alleviation of Stresse

Enhancement of seedling growth under biotic and abiotic stresses

Azospirillum found protecting tomato seedlings from bacterial speck by out-competing and displacement of this foliar pathogen. Bashan and de-Bashan [45] reported suppression of foliar bacterial speck pathogen *Pseudomonas syringae* pv. tomato by *A. brasilense*. In this case, the bacterial speck pathogen

is a foliar pathogen and *A. brasilense* is rhizosphere inhabiting microorganism, both are having different niches. In the study, application of *P. syringae* on rhizosphere with *A. brasilense* or foliar application of *A. brasilense* with *P. syringae*, suppresses the disease development. To maintain the population of *A. brasilense*, which is not a regular inhabitant of phyllosphere, several applications of buffered malic acid in a low concentration have done and it found decreasing the population of *P. syringae* pv. tomato to almost undetectable levels. It improved plant growth similar to uninoculated healthy control plants.

Azospirillum enhances seedling growth in both normal and draught conditions. It can even work in the presence of tebuconazole, fungicide widely used in wheat. The characteristic *Azospirillum* enhancing effects observed on roots remained unaltered by Tebuconazole [46].

Alleviating plant from water stress

Azospirillum inoculation found improving water status sorghum plants under water stress. Inoculated plants showed more water in their foliage, leaf water potential and lower canopy temperature than uninoculated plants. Soil moisture content in soils of *Azospirillum*-inoculated plants was greater and water was extracted from deeper layers in the soil profile. Yield increase in sorghum inoculated with *Azospirillum* was attributed primarily to improved utilization of soil moisture [13].

Increases photosynthetic pigments

Experiment conducted in wheat seedlings with *Azospirillum brasilense* Cd in alginate bioformulation found increasing the quantity of several photosynthetic pigments significantly. Apart from chlorophyll a and b, auxiliary photoprotective pigments like violaxanthin, zeaxanthin, antheroxanthin, lutein, neoxanthin, and β -carotene also enhanced by *Azospirillum*. It form during stress in the plant and protects the photosynthetic machinery [47]. The cause behind this mechanism can be attributed to several effects of *Azospirillum* additively like hormonal influence, increased water and mineral uptake, changes in membrane function and many more [48].

Revegetation of mangroves in nutrient deficient environment

Mangroves are trees and large vegetations very crucial as marine coastal habitats. It is very important from biodiversity point of view. In semi arid tropics, the mangroves hardly regenerate after clear cutting, because salt stress in seedlings inhibits its establishment. There is also a problem of poor nutrients in soil. *Azospirillum brasilense* and *Azospirillum halopraeferens* can colonize the roots of mangroves, survive in high salt concentration and improves the growth of host plant. Once seedling established, it can resist high salt concentration and grow normally [30].

Restoration of vegetation in desert:

Cardon cactus (*Pachycereus pringlei*), the giant columnar cactus grows with a nurse tree which reduces the environmental stress and establishes the disturbed arid soils. Legume is a good facilitator for cardon cactus. The use of microbial inoculants like mycorrhiza was also tried to help early plant growth but mycorrhiza can't infect this plant at early stage of growth. *Azospirillum* enhances root and shoot growth of cardon cactus at early stage when it is not colonized by the mycorrhiza. By this, nurse trees can be replaced by *Azospirillum*. It can work as a key in artificial restoration process in deserts and reduce soil erosion [49].

Other Benefits

Enhancement of seed quality in plants

Bashan *et al.* [50] inoculated halophytic oilseed *Salicornia bigelovii* Torr. with eight species of halotolerant bacteria including *Azospirillum halopraeferens*, a mixture of two *Azospirillum brasilense* strains. These plants were grown seawater-irrigated pots in environmental conditions similar to the native ones. These bacterial species showed significant increase in plant growth and other parameters. The N and protein content of the plant foliage was significantly reduced but the N and protein content of seeds significantly increased by the inoculation. Similar trend was observed in P content of plants. The mixture of *A. brasilense* strains showed higher total lipid content of foliage. In three treatments palmitic acid in seeds was significantly increased.

In a study by Stefan *et al.* [51] inoculation of *Azospirillum* to the plants have a positive effect on the total soluble protein, carbohydrates and lipids content of soybeans. Although, there are no significant differences between inoculated and uninoculated plants in soluble reducing carbohydrate content, lipid content and relative humidity. But, the soluble protein content per gram in inoculated plants is significantly greater than uninoculated plants.

Bacterial Nitrate reductase activity

Nitrate assimilation is a process by which reduced nitrogen is accumulated in the plants. It occurs in two different steps; first, nitrate is reduced to nitrite by nitrate reductase (NR); and in second step, nitrite is reduced to ammonia by nitrite reductase which is then assimilated by the plants [52]. Under water stress conditions, plant NR activity declines which results in low nitrate absorption by the plants [53]. It found reducing nitrate assimilation in wheat [54] and cause poor plant growth.

Lahiri [55] reported that the adverse effects of water stress condition can be alleviated by using

nitrogenous fertilizers. *Azospirillum lipoferum* was described [56] to have nitrate reductase (NR) activity in *Azospirillum* and proven to have relationship with nitrogenase activity. Presence of nitrate due to nitrate reductase activity simulates anaerobic N_2 ase activity. Studies conducted with mutants of NR concluded that inoculation with *Azospirillum* spp. Having NR activity enhances the plant growth as it affects the N_2 ase activity [57]. This indicates that the nitrogen nutrition by the *Azospirillum* spp. is because of N_2 ase and NR activity together. Application of *Azospirillum* also enhances nitrogen nutrition of the plants and reported to alleviate plant from stress [59]. Although, it is not fully understood that whether bacterial NRA, bacterial induced plant NRA or an interaction of both are responsible for enhanced assimilation of soil nitrogen.

Increases the potential of microalgae

There are many roles an organism plays in the ecosystem by interacting others. A positive and synergistic relation is beneficial for both organisms. In case of unicellular algae *Chlorella vulgaris* and *Azospirillum brasilense* immobilized in alginate beads, it increases the population of both organisms. It is useful in waste water treatment. It creates a biofilm which is strong enough in holding both species together [60]. This kind of feature is very crucial where attachment to the surface is rather difficult.

Doesn't cause hypersensitive response to plant

Activation of hypersensitive response is a key feature of a pathogen. *Azospirillum* doesn't found activating hypersensitive response in plants and thus it is safe to be used in plants. However, there are reports that all *Azospirillum* strains increased moderately the phytoalexins production in disease-resistant cultivars, but significantly lower than compatible pathogens [61].

CONCLUSION

There is huge demand for a successful bio-fertilizer, and *Azospirillum* is a potential candidate. It promotes plant growth by different mechanisms and may also have many other beneficial impacts indirectly to the plants. Efforts for its popularization in non-traditional use (more than just a nitrogen fixing microbe) are required. Research can also be taken up in enhancing efficiency of *Azospirillum* in harsh conditions like draught, salinity, heat, flood, etc. so that the inoculation effect can be translated in to yield in changing climate also.

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