**Mini Review**

Harnessing wheat genotype x *Azobacter* strain interactions for sustainable wheat production in semi arid tropics

Rishi Kumar Behl¹, Neeru Narula², Manjula Vasudeva³, Atsuya Sato⁴, Takuro Sihnano⁵ and Mitsuru Osaki⁶

¹ Department of Plant Breeding, CCS Haryana Agricultural University, Hisar-125004, India
² Department of Microbiology, CCS Haryana Agricultural University, Hisar-125004, India
³ Department of Genetics, CCS Haryana Agricultural University, Hisar-125004, India
⁴ Department of Plant Nutrition, Hokkaido University, Sapporo, Japan
⁵ Creative Research Initiative ‘Sousai’, Hokkaido University, Sapporo, Japan

**INTRODUCTION**

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. Moreover, it is a staple food for nearly 35% of the world population. The expected global demand for wheat in the year 2020 will be from 840 to 1,050 million tons (Rosegrant *et al*., 1995). To meet this growing demand, the global average grain yield must increase from the current 2.5 t ha⁻¹ to 3.8 t ha⁻¹. Wheat yield *per se* is closely associated with input responsiveness. With the advent of Mexican semi-dwarf high yielding wheat varieties, a laudable increase in yield was realized in several conventional wheat belts in the mid-sixties (Rajaram, 1999). This could not have happened without the heavy application of fertilizers. The manifold increase in the doses of NPK fertilizers, generally devoid of micronutrients, resulted in a rapid depletion of soil micronutrients.

Continuous use of high doses of chemical fertilizers has adversely affected soil health leading to a decline in productivity in many wheat growing areas in the world. In addition, there are large areas in Asia and Africa where wheat is grown under rainfed and/or limited water supply conditions. In these areas, soils are poor in mineral nutrients, organic carbon and rhizospheric activity. Due to economic reasons and associated risk factors, fertilizer application rates there are far below the recommended doses. This contributes to low productivity of wheat in such areas. Therefore, to maximize production all over the world emphasis is being placed on the selection of high and low input efficient wheat genotypes responsive to bioinoculants and applied inorganic nutrients for different agro-climatic areas.

*Azobacter chroococcum* is a gram negative bacterium belonging to family Azotobacteraceae, a coherent group of aerobic, free living diazotrophs able to fix atmospheric nitrogen in nitrogen free or nitrogen poor media with organic carbon compounds as an energy source. Several properties of *Azobacter* are considered to be responsible for their beneficial effects. These include:

1) Ability to produce ammonia, vitamins and growth substances that enhance seed germination (Brown, 1975; Narula *et al*., 1981; Narula & Tauro, 1986).

2) Production of IAA and other auxins, such as gibberellins and cytokinins (Martinez-Toledo *et al*., 1988; Neito & Frankenberger, 1989; Verma *et al*., 2001) which enhance root growth and aid in nutrient absorption.

3) Inhibition of phytopathogenic fungi through antifungal substances (Sharma & Chahal, 1987; Verma *et al*., 2001).

4) Production of siderophores (Neiland, 1981; Knosp, *et al*., 1984; Page & Huyer, 1984; Page, 1987; Suneja & Lakshminarayana, 1993; Suneja *et al*., 1996) which, solubilize Fe⁺⁺ and suppress plant pathogens through iron deprivation. *Azobacter vinelandii* is also reported to have the ability to synthesize siderophores under Fe deficient conditions (Tindale *et al*., 2000).

Biofertilizers are products formulated from living cells of different types of microorganisms that have the ability to mobilize nutrients from a non-useable to a usable form through biological processes. These microorganisms come from the soil biosphere and are therefore already part of the plant’s natural environment. Broadly, the microorganisms that are in use as wheat biofertilizers include the free living and associative nitrogen fixing and phosphate solubilizing rhizobacteria and the mycorrhizal fungi that are capable of mobilizing unavailable nutrients from the soil and transporting them into plant roots. Recently, to these have been added, the plant growth promoting rhizobacteria (PGPR), which stimulate plant growth and repress root diseases by a variety of mechanisms.
Azotobacter as a Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth regulators usually are defined as non-nutrient organic compounds, either natural or synthetic, that affect the physiological processes of growth and development in plants when applied in low concentrations. The term ‘plant hormone’ or ‘phytrogen’ is restricted to naturally occurring substances and includes four main groups of compounds: auxins, cytokinins, gibberellins (GAs) and abscisic acid (ABA).

Rhizosphere-colonizing bacteria, including Azotobacter chroococcum, that possess the ability to enhance plant growth when applied to seeds, roots or tubers are called plant growth-promoting rhizobacteria (PGPR) (Kukreja et al. 2004). PGPR was first defined by Kloeper & Schroth (1978) to describe soil bacteria which, when used as an inoculant, enhance plant growth. Narula et al. (2005) reported phytohormones produced by Azotobacter chroococcum strains (both identified and unidentified) and Pantoea agglomerans. They further observed that plant responses to inoculation with PGPR include enhanced nitrogen fixing ability with minimal inoculation, direct increases of various growth parameters (plant dry weight, development and morphology of root system, grain yield, protein and mineral nutrient content), displacement of deleterious and pathogenic rhizosphere microorganisms, increased phosphorus solubilization and enhanced VA-mycorrhiza. Other proposed mechanisms may also be involved in plant growth stimulation, mainly improvement of water and mineral uptake (Bashan and Levanony 1991; Bertrand et al. 2000) and production of biologically active substances, such as vitamins, amino acids, phytohormones (Garcia de Salamone et al. 2001; Glick 1995; Persello-Cartieux et al., 2003) and antibiotics (Giacomodonato et al., 2001).

Verma et al. (2004) conducted research on the comparative performance of phytohormone producer and non-producer strains of Azotobacter chroococcum on wheat. Results suggest that the strains capable of producing two phytohormones, along with application of third phytohormone, had synergistic effects on plant growth. When all the three phytohormones were supplied exogenously with the non-producer strain, the non-producer strain could not compete with the strain that produced all three phytohormones. This indicates that plant growth promotion is caused by the cumulative effects of more than one factor. Researchers observed similar results (Lippman et al., 1995), although the specific mechanisms are not well understood yet (Glick, 1995; Kloeper, 1993). These researchers’ results suggest that Azotobacter strains secrete sufficient quantities of phytohormones required for better plant development. Plant growth response to Azotobacter inoculation could not be attributed to nitrogen fixation alone, but phytohormones also have a significant role in plant growth promotion. Since exogenously applied phytohormones without Azotobacter inoculation could not mimic the growth effect, it is suggested that other characteristics present in Azotobacter have some role in plant growth stimulation. So, plant growth promotion by Azotobacter inoculation may be due to synergistic effects of several factors.

Azotobacter as an N₂ fixing biofertilizer

Microorganisms that fix nitrogen are called diazotrophs. Those microbes that fix nitrogen independent of other organisms are called free-living bacteria. And those bacteria fixing N₂ for nitrogen input to the biosphere are also considered PGPR (Postgate, 1998). Using a ¹⁵N dilution technique that measures δ¹⁵N values to estimate which plants fix atmospheric N₂ by rhizosphere-associated bacteria is one of the most direct methods to evaluate the contribution of those bacteria. However, the results are rather puzzling, because it is very difficult to identify the presumed non-N₂-fixing plants. Lee et al. (1994) analyzed the δ¹⁵N values of pearl millet grains with and without Azospirillum lipoferum inoculation and sorghum with and without Azotobacter chroococcum inoculation, and found no significant differences in δ¹⁵N values of the plants with and without associative N₂-fixing bacteria inoculation. The use of microbial inoculants, often called biofertilizers, has increased gradually over the past two decades in India. The researches farmers have turned to biofertilizers are many, the most important one being the high cost of chemical fertilizers.

Azotobacter is capable of converting nitrogen to ammonia (Newton et al., 1953; Bishop et al., 1982), which in turn is taken up by the plant. Mutant strains that have nitrogenase synthesis and are blocked in their ability to utilize ammonia, excrete ammonia ions into the medium. Nitrogen fixation is highly regulated in all microorganisms that have been studied (Merrick, 1992), and its rate may depend on environmental conditions. Nitrogen fixation activity (nitrogenase) is repressed by ammonium in Azotobacter spp. Therefore, the presence of high amounts of nitrogenous fertilizer in the field reduces their effectiveness as biofertilizers. It is important to isolate derepressed mutants for agronomic use and develop them in order to offer a new approach in the field of biofertilizers. There are reports of isolated mutants...
that can fix nitrogen in the presence of ammonium and excrete ammonia (Gordon & Brill, 1972; Gordon & Jacobson, 1983; Bela et al. 1986). Constitutive mutants that overproduce nitrogenase and excrete ammonia in nitrogen free un-supplemented medium, suggest that they could be better bio-fertilizers. Bela et al. 1986 isolated mutants resistant to analogues of ammonia viz., methyl alanine (mal), methionine sulfoximine (MSX) and methyl ammonium chloride (Mac). Derepressed nitrogenase activity and ammonia excretion was studied in all the mutants however Mac mutants showed higher nitrogenase but no derepressed nitrogenase activity. All these classes of mutants showed early ammonia excretion.

Lakshminaryana et al. (2000) isolated and tested mutants of Azotobacter strain A 103, resistant to the three metabolic analogues (Msx, Mal and Mac), for ammonia excretion and nitrogen fixation in presence of ammonium. The parent strain A103 did not express ARA in Burk’s medium supplemented with ammonium acetate and showed ammonia excretion only after 9 days of incubation under stationary conditions. Msx resistant mutants showed nitrogenase activity in ammonium acetate supplemented medium. All of these mutants exhibited early ammonia excretion. The mutant Msx 21, which showed slightly more ARA than the parent strain (A 103), and Msx 1, which showed less ARA in Burk’s medium, expressed more ARA in ammonium acetate supplemented medium compared to two other mutants, Msx 27 and Msx 37. Maximum ammonia excretion (6.4 to 7.0 µg ml−1) was observed in the mutants Msx 1, Msx 21 and Msx 26 at 2 days of incubation. At 9 days of incubation, mutants Msx 1, Msx 27 and Msx 37 showed more ammonia excretion, ranging from 7.9 to 10.2 µg ml−1. Among methyl alanine-resistant mutants, Mal 24 and Mal 27 mutants showed maximum derepressed ARA (0.8 to 1.0 µg ml−1 C,H, reduced/hr/µg protein). Mutants Mal 1 and Mal 27 showed more ammonia excretion compared to other mutants at 2 days of incubation, whereas Mal 24 and Mal 30 showed a 68 to 120% increase in ammonia excretion over the parent strain A103 at 9 days of incubation.

Derepressed nitrogenase activity in the presence of ammonium acetate was not observed in methyl ammonium chloride-resistant mutants. However, mutant Mac 63 showed more ARA than the wild type parent strain. Two mutants Mac 27 and Mac 63 excreted 5.6 and 5.2 µg ml−1 ammonia at 2 days of incubation and showed 128 to 154% increase in ammonia excretion over the parent strain at 9 days of incubation.

The selected mutants of A. chroococcum, along with parent strain A103, were tested as inoculants under field conditions on wheat (Lakshminaryana et al. 2000). Parent strain A103 was found to increase the grain yield of wheat (16.3%) compared to uninoculated controls. Overall grain yield increased in wheat inoculated with the analogue-resistant mutants by 1.2–30% compared to parent strain inoculated plants. The results also indicate a host dependent response on grain yield due to inoculation of various analogue-resistant mutants. For example, two Msx-resistant mutants, Msx 26 and Msx 37 showed only a 1.2 and 5.0% increase in grain yield on wheat, respectively, but other mutants, Msx 1, Msx 27, Mac 30 and Mac 27, were found to increase the grain yield of wheat (varying from 5.0 to 15.0%) compared to the parent plants.

**Azotobacter as a Phosphate Solubilizing Microorganism**

Azotobacter, like other soil microorganisms, plays a significant role in mobilizing P from the native soil P pool, as well as from added insoluble phosphates such as rock phosphates, for plants to use. The rates of solubilization vary with the inorganic P source and the Azotobacter strains involved. Solubilization of tricalcium phosphate in agar medium has been used as an initial criterion for isolation and enumeration of P solubilizing strains. Organisms growing on such media are able to solubilize P and produce a clear zone around themselves due to the dissolution of fine particles of tricalcium phosphate. The phosphate solubilizing Azotobacter strains may produce effective solubilizing agents, such as organic acids or chelating substances, in microenvironments in the vicinity of rock phosphate or in the rhizosphere, for example. Under these conditions P is converted to an available form.

Kumar & Narula (1999) evaluated Phosphate-solubilizing strains of A. chroococcum isolated from the wheat rhizosphere for their ability to produce indole-acetic-acid (IAA) and solubilize tricalcium phosphate (TCP) and Mussoorie rock phosphate (MRP). Strains were selected on the basis of the clearance zone on solid agar media of Pikovskaya and Jensen’s media containing TCP, and phosphate solubilization in Jensen’s liquid culture medium (Jensen, 1951) containing both TCP and MRP. Mutants of the best phosphate-solubilizing (TCP 1.52 µg ml−1, MRP 0.19 µg ml−1), IAA-producing A. chroococcum strain P4 were developed and screened for P solubilization and phytohormone production.

Five mutants solubilized more P (in the range of
Proton release is thought to be the main mechanism that increases phosphorus availability (Illmer & Schinner, 1995; Villegas & Fortin, 2002). However, other phosphate solubilizing substances should also be included for consideration (Bajpai & Sundara Rao, 1971; Banic & Dey, 1981; Whitelaw, 2000; Staunton & Leprince, 1996).

Kumar et al. (2001a) conducted a pot experiment in a

| Table 1. Effect of *Azotobacter chroococcum* inoculation on spike traits, yield, total nitrogen and viable counts in C591/CS disomic substitution wheat lines in different soil types |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Heading days    | Tiller number   | Spike weight (g) | Spikelets /spike | Grain no./ spike | Yield (g)        |
| **A Treatments** |                 |                 |                 |                 |                 |                 |
| Control        | 122.427         | 1.757           | 0.725           | 13.254          | 22.038          | 0.97            |
| Mac 27         | 119.427         | 2.081           | 0.947           | 15.208          | 25.614          | 1.562           |
| M 37           | 119.308         | 2.236           | 1.058           | 15.655          | 27.795          | 1.96            |
| C. D.          | 1.147           | 0.094           | 0.029           | 0.313           | 0.644           | 0.084           |

| **B Soil Types** |                 |                 |                 |                 |                 |                 |
| Clay            | 119.009         | 2.554           | 1.071           | 16.737          | 29.611          | 2.147           |
| Sand            | 121.412         | 1.495           | 0.75            | 12.674          | 20.687          | 0.847           |
| C. D.           | 0.936           | 0.077           | 0.024           | 0.256           | 0.526           | 0.069           |

| **C Genotypes** |                 |                 |                 |                 |                 |                 |
| C591            | 115.894         | 2.044           | 1.068           | 13              | 21.611          | 2.993           |
| CS              | 125.25          | 2.233           | 0.908           | 14.056          | 27.611          | 2.045           |
| CH1A            | 115.906         | 2.533           | 1.143           | 15.417          | 32.111          | 2.174           |
| CH1B            | 113.7           | 1.611           | 1.028           | 14.167          | 28.833          | 1.322           |
| CH1D            | 115.761         | 2.022           | 1.061           | 14.917          | 26.111          | 1.798           |
| CH2A            | 116.617         | 2.2             | 0.998           | 15.389          | 23.278          | 1.638           |
| CH2B            | 115.833         | 1.644           | 1.233           | 15.528          | 28.389          | 1.49            |
| CH2D            | 113.7           | 1.85            | 0.876           | 12.75           | 21.056          | 1.505           |
| CH3A            | 119.817         | 2.094           | 0.923           | 14.722          | 26              | 1.398           |
| CH3D            | 119.111         | 2.406           | 0.585           | 11.972          | 18.5            | 1.139           |
| CH4A            | 121.367         | 1.9             | 0.728           | 15.361          | 23.944          | 1.02            |
| CH4B            | 123.506         | 1.694           | 0.859           | 15.611          | 25.278          | 1.179           |
| CH4D            | 117.978         | 1.956           | 1.049           | 16.3            | 30.944          | 1.326           |
| CH5A            | 123.178         | 2.489           | 0.614           | 15.167          | 19.611          | 1.743           |
| CH5B            | 120.833         | 1.75            | 0.656           | 12.583          | 21              | 0.935           |
| CH5D            | 119.889         | 1.9             | 0.805           | 13.694          | 24.111          | 1.239           |
| CH6A            | 123.122         | 2.072           | 1.125           | 17.028          | 30.444          | 1.529           |
| CH6B            | 123.689         | 2.083           | 0.855           | 15.639          | 24.278          | 1.308           |
| CH6D            | 122.567         | 2.111           | 0.921           | 16.278          | 24.389          | 1.417           |
| CH7A            | 123.633         | 1.906           | 0.956           | 14.583          | 25.222          | 1.219           |
| CH7B            | 131.256         | 2.144           | 0.676           | 14.639          | 20.278          | 1.047           |
| CH7D            | 124.789         | 1.9             | 0.956           | 14.528          | 30.278          | 1.476           |
| C. D.           | 3.105           | 0.255           | 0.078           | 0.848           | 1.743           | 0.228           |

1.5–1.7 µg ml⁻¹ of TCP and 0.19–0.22 µg ml⁻¹ of MRP) than the parent strains. *In vitro* growth emergence studies of three wheat varieties, viz. C-306, WH-542 and HD2009, showed better performance with phosphate-solubilizing mutants than with the parent strain. Deubel & Merbach (2005) also demonstrated the contribution of *A. chroococcum* on the solubilization of calcium phosphates.
green house to investigate the establishment of phosphate solubilizing strains of *Azotobacter chroococcum*, including soil isolates and their mutants, in the rhizosphere and their effect on growth parameters and root biomass of three genetically divergent wheat cultivars (*Triticum aestivum* L.). Five fertilizer treatments were performed: Control, 90 kg N ha⁻¹, 90 kg N + 60 kg P₂O₅ ha⁻¹, 120 kg N ha⁻¹ and 120 kg N + 60 kg P₂O₅ ha⁻¹. Phosphate solubilizing and phytohormone producing parent soil isolates and mutant strains of *A. chroococcum* were isolated and selected with an enrichment method. *In vitro* phosphate solubilization and growth hormone production by mutant strains increased compared to soil isolates. Seed inoculation of wheat varieties with P solubilizing and phytohormone producing *A. chroococcum* showed better response compared to controls. Mutant strains of *A. chroococcum* showed a higher increase in yields of grain (12.6%) and straw (11.4%) than the control. Mutant strain M37 performed better in all three varieties in terms of increase in grain yield (14.0%) and root biomass (11.4%) than the control.

Inoculation of wheat varieties with soil isolates and mutants of *Azotobacter chroococcum* led to a greater uptake of NPK with mutant strains bioinoculation. The P uptake (mg plant⁻¹) in control was 7.92 in parent isolates, while it was 9.20 in mutant strains. Also, after 60 days of inoculation the survival rate of mutants was higher (12-14%) in the rhizosphere than the parent soil isolates in rhizosphere of variety C306.

**Azotobacter: siderophore production for disease control**

Siderophores are low molecular weight compounds that are produced under iron limiting conditions, chelate the ferric iron (Fe³⁺) with a high specific activity and serve as a vehicle for the transport of Fe (III) into microbial cells. Siderophores are also known to bind other metals, such as Mn, Cr, Ga and Pu. (Birch & Bachofen, 1990). Siderophores are also known to act as growth factors and as phytopathogenic suppressive agents (Calvente et al., 2001). *Azotobacter chroococcum* is known to produce siderophores (Knosp et al., 1984; Suneja et al., 1996). Antagonistic action of *A. chroococcum* on phytopathogens has been studied by various researchers (Schroth & Hancock, 1982; Meshram & Jager, 1983; Weller, 1988; Verma et al., 2001), but whether this inhibition is due to siderophores or antifungal properties was not clear. Beniwal et al. (1996) conducted extensive field experiments to evaluate the effect of *A. chroococcum* strains/mutants on the incidence of flag smut. The results revealed that flag smut incidence in wheat was significantly less under bioinoculation compared to the control. The maximum disease reduction was in strain Mac 21 (37.96 %) followed by strains 6(2) (34.18%); the lowest was 14% in Mac 57. An increased incidence of flag smut in some strains namely, Mac 54, Mac 68 and in parent isolate 103, was also observed.

Bansal et al. (1999) determined the effects of rhizospheric bacteria on plant growth of wheat infected with *Heterodera avenae* in potted plants. Out of four rhizospheric diazotrophs tested, *A. chroococcum* (HT 54) showed maximum reduction in nematode infection (48%) followed by *Pseudomonas* (11%) and *Azospirillum* (4%). In contrast, *R. ciceri* was totally ineffective. Although *Pseudomonas* was more effective in mitigating nematode infection than *Azospirillum*, the biomass accumulation by wheat was better when inoculated with *Azospirillum*.

**Azotobacter as an endophyte**

There would be a great competitive advantage for both the nitrogen fixing bacteria and the plants if a more intimate internal association could be established. Many soil bacteria have demonstrated the potential to promote plant growth and enhance plant yields. *A. chroococcum* is known to produce indole acetic acid, gibberellic acid and cytokinins (Crozier et al., 1988; Bottini et al., 1989; Cacciari et al., 1989). Application of natural (microbiologically produced by plant growth promoting regulators) or synthetic plant growth regulators is often recommended to improve plant quality and yield as well as to alter plant life processes. Treatment with auxins has shown to increase the colonization of roots by soil bacteria, *e.g., Azospirillum* (Tchan et al., 1991; Kennedy & Tchan, 1992). A change in morphology of roots to form paranodules has been reported following the addition of auxin analogues. Sriskandarajah et al. (1993) and Dobbelare et al. 1999 reported these structures with *A. brasilense* (Sp7) partly due to the development of a protected niche. These paranodule structures derived from the induction of the initials of the lateral roots are quite dissimilar to root nodules, particularly when colonized by non-symbiotic bacteria.

With a 2,4-D treatment, paranodules were also formed on rice root (Nilsson, 2002). Koval’skaya (2001) reported that paranodules were formed by *Micrococcus* sp. + *Rhodococcus* sp. isolated from the rhizosphere of the cycad *Cycas revoluta* inoculation. Narula et al. (2005) evaluated soil bacteria belonging to the genus *Azotobacter, Pantoea* and some unidentified isolates *in vitro* for phytohormone production. The German
wheat variety Munk was inoculated with several soil bacteria with exogenously applied hormones (IAA, 2,4D) and a flavonoid (naringenin) and half the amount of recommended doses of fertilizers under green house conditions. Analyses of root exudates and in vitro phytohormone production by various bacterial isolates were also carried out. Compared to the uninoculated and untreated controls, most of the treatments with bacteria and hormones had thick roots, decreased root length and increased root hairs in Petri dishes, as well as soil. In the case of IAA producing bacteria addition of 10 µg mL\(^{-1}\) IAA, thereby helps in the formation of paranodules. One or two big paranodules were observed. Researchers were able to re-isolate the organism from the paranodules and obtain the same results. Gopalsamy et al. (2000) reported that the addition of naringenin can enhance xylem colonization.

Narula et al. (2005) did not demonstrate nitrogen fixation by these paranodules since their focus was on establishing an efficient colonization and observing changes in root morphology. However, Yu and Kennedy (1995) observed nitrogenase activity in 2,4D induced root structures of *Azorhizobium*. Kennedy et al. (1997) reported that 10\(^7\) azospirilla in the wheat seedlings are actively fixing nitrogen. Sabry et al. (1997) showed that wheat grown in pots and inoculated repeatedly with *A. caulinodans* colonized tissues at the point of emergence of lateral roots and appeared to contribute significant amounts of nitrogen fixation to the plant.

**Azotobacter chroococcum effect on wheat production**

*Azotobacter chroococcum* has been widely tested for its stimulat ing effects on wheat in pot and field experiments. **Effect on roots**: *Azotobacter* in the root zone of wheat plants is known to improve the nitrogen feeding of plants in soil, especially when their propagation is stimulated. *Azotobacters* in the rhizosphere have beneficial effects on wheat roots, including:

i) Increased water uptake, because the additional microbial biomass of *Azotobacter*, along with other natural occurring microflora, can hold water in close proximity to plant roots. *Azotobacter chroococcum* synthesizes poly-β hydroxyl butyrate during stress conditions. This biomolecule is mainly responsible for plant’s ability to overcome unfavorable conditions and it also helps hold water molecules near the rhizosphere (Page 1987, Page 1992, Parshad et al. 2001).

ii) Production of growth factors (e.g. vitamins, auxins and gibberellins) which increase seed germination, root hair development, and overall growth, like the rhizosphere of wheat seedlings. So, it is not only *Azobacter* contribution to nitrogen fixation, but also its production of growth regulators that beneficially effects plant growth. (Lippman et al. 1995; Pathak et al. 1995; Verma et al. 2004). It is known that in addition to other functions, auxins control root formation and relax the cel l wall and gibberellins increase leaf and root growth. All these activities may be related to the formation of an extensive root system, thereby enhancing plant growth and increasing leaf size subsequently aiding endophytes’ access to the photosynthesizing nutrients.

iii) Increased availability of nutrients (recycling/solubilization). This effect may be related to hormone production by *Azotobacter*. These substances are known to increase the number of root hairs, lateral root and root mass. Hence, the absorptive capacity of the root system for nutrients is expanded directly due to enhanced root growth or indirectly due to the presence of more sites created for VAM.

iv) Ammonia excretion: Diazotrophs like *Azotobacter* are capable of converting nitrogen to ammonia (Lakshminaryana et al., 2000) which in turn is taken up by the plant. Derepressed strains of *Azotobacter* excrete large amounts of ammonia. These strains have no special requirement and excrete ammonia when supplied with a variety of carbon sources.

**Effect on Plant Growth**: Significant increases in wheat grain yield, number of tillers and dry matter accumulation and NPK uptake has been reported by Narula et al. 2000 and Kumar, et al., 2001b. *Azotobacter* seed-inoculation was tested in a field trial in North-India with ten Indian wheat cultivars (Manske et al., 1998; Manske et al., 2000). Significant effects of the varieties and bioinoculants were observed. The effect of inoculation was more marked in the root than in the shoot. The total root length increased. However, the response of grain yield to *Azotobacter* was plant genotype dependent and appeared to be related to improved P and N utilization efficiency. Phosphate utilization efficiency in grain yield production was increased (average 13%) more than N utilization efficiency (5%). Furthermore, N uptake was not qualitatively improved by *Azotobacter* inoculation. This supports the hypothesis that *Azotobacter* aides plants by producing phytohormones, which stimulate root growth and VAM infection, rather than by fixing nitrogen.
Inoculation of *Azotobacter chroococcum* (Aze) also compliments wheat-AMF interaction due to nitrogen fixation, phytohormone production and phosphate solubilization properties (Narula *et al.*, 1981; Kumar *et al.*, 2001). Plant growth promoting rhizobacteria, such as *Azotobacter chroococcum*, produce hormones and other growth promoting substances. As such they are helpful in promoting plant growth and mitigating nutrient stress by solubilizing fixed phosphorus.

**Genetic Variability in Wheat and Response to Azotobacter**

Wheat’s response to inoculation of *Azotobacter varies* by genotype (Singh *et al.*, 2002; Behl *et al.*, 2003). Such variation is generally attributed to variability among wheat genotypes for root characters, vernalization and photoperiod response genes that are associated with tillering, root characters, nutrient uptake, stay green, photosynthesis duration and efficiency etc. (Manske *et al.*, 2000).

In this context, Singh *et al.* (2004) evaluated 20 spring wheat genotypes' response to *Azotobacter chroococcum* under medium fertility (90 Kg N and 60 Kg P₂O₅) field conditions. In general, the performance of various genotypes was better with *Azotobacter* inoculation than without. Out of 20 genotypes, 11, namely, TD, TD(E), WH157, WH147, WH147M, C59L, C306M10, PBW175, SG8809, SG70 and TWT49, had significantly higher biological yields per tiller when inoculated than not. Likewise, 8 genotypes, namely, WH 157, WH 147M, C306M10, Raj 3765, PB 175, SG 8809, SG 170 and Thatcher, recorded significantly higher grain yield spike⁻¹ with *Azotobacter* inoculation. The genotypes WH 157, C306M10 and SG 8809 responded favorably for all the four characters. However, significant increases in spike productivity following inoculation was either due to improvement in grain weight (Thatcher, SG70) or grain number spike⁻¹ (WH147M, PBW175, Raj 3765) or both traits (WH157, C306M10, SG8809). These results show that the different genotypes' traits studied are affected differently by *Azotobacter* inoculation, possibly due to variable plant-microbe interactions.

**Localization of Response Genes on Different Wheat Chromosomes**

A pot experiment was conducted by Vasudeva *et al.* (2002) to evaluate the responses of 20 disomic chromosome substitution lines of cv. C591 in the background of Chinese spring to inoculation of two strains of *Azotobacter*, M37 and Mac 27, grown under two different soil types, sand and clay-loam. The experiment was laid out in a completely randomized block design (CRD) with three replications. Observations were recorded on important seedling, root and plant traits that contribute to grain and biological yield.

Overall, most lines responded better to M37 than Mac 27 (Table 1). Between soil types, clay loam supported the highest number of *Azotobacter* colonies in wheat rhizosphere. This may be the main reason that various disomic substitution lines, particularly 1D, 2B and 1A, have better performance with regard to yield and yield attributing traits and high nitrogen content. Among the disomic substitution lines, 3A, 5A, 2B, 7B and 1D had several improved seedling, root and plant traits and hence better grain and biological yield. As several lines responded to inoculation by M37 in particular, it is expected that the response is due to growth promoting phytohormones produced by bio-inoculants and the responsive genes (QTLs) localized on different chromosomes. Chromosome specific selective breeding is recommended to concentrate gene constellations on one or two chromosomes to facilitate wheat breeding for organic regimes.

Singh *et al.* (2005) evaluated wheat genotypes C591 and Chinese Spring and twenty disomic chromosome substitution (DCS) lines of C591 in the background of Chinese Spring to learn about genotypic variability and to localize genes/QTLs governing uptake and response to micronutrients when inoculated with *Azotobacter chroococcum*. The experiment replicated thrice was laid out in a completely randomized design with three treatments: T₁ (recommended doses, 120Kg N, 60KgP.O₅ and 60Kg K.O); T₂ (NPK+ micronutrients viz. Fe, Cu, Zn, Mn (recommended doses) and T₃ (NPK+ micronutrients + *Azotobacter chroococcum* (strain M37)). Observations were recorded on content and uptake of micronutrients (Fe, Cu, Zn, Mn) and viable counts of *Azotobacter* in rhizosphere.

The results revealed that the performance of the parents and 20 disomic lines was significantly superior when treated with NPK+ micronutrients + *Azotobacter* (T₃) than with NPK+ micronutrients (T₁) and that of T₃ over T₁. Uptake (µg/g) of Fe, Mn, Zn, Cu, were the highest in ID DCS line, while 6B, 7B and 5B DCS lines were on par with the ID DCS line. Grain yield plant⁻¹ was significantly and positively correlated with Mn (0.652), Zn (0.585), Fe (0.552) and Cu content (0.427). Significant differences were observed in viable counts of *Azotobacter* in the rhizosphere of different DCS lines and their parents. At 80 days after sowing, maximum viable counts were...
Table 2. Mean performance of five wheat genotypes with (A) or without Azotobacter inoculation (C) under rainfed field conditions.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Tillers plant−1</th>
<th>Plant height (cm)</th>
<th>Spikelets spike−1</th>
<th>Biological yield plant−1 (g)</th>
<th>Grain yield plant−1 (g)</th>
<th>100–grain weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WH147M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10.6</td>
<td>82.5</td>
<td>20.6</td>
<td>45.3</td>
<td>16</td>
<td>4.26</td>
</tr>
<tr>
<td>A</td>
<td>11.6</td>
<td>85.3</td>
<td>24.3</td>
<td>48.4</td>
<td>19</td>
<td>4.33</td>
</tr>
<tr>
<td>% increase</td>
<td>9.43</td>
<td>3.39</td>
<td>17.96</td>
<td>6.84</td>
<td>18.75</td>
<td>1.64</td>
</tr>
<tr>
<td>WH533</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>80</td>
<td>19.3</td>
<td>52.6</td>
<td>26.3</td>
<td>4.7</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>81.3</td>
<td>20.3</td>
<td>53.6</td>
<td>25.8</td>
<td>4.7</td>
</tr>
<tr>
<td>% increase</td>
<td>9.09</td>
<td>1.62</td>
<td>5.18</td>
<td>1.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PB W 175</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>90.6</td>
<td>17.3</td>
<td>23.6</td>
<td>10.3</td>
<td>5.06</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>91.6</td>
<td>21</td>
<td>28.3</td>
<td>12.6</td>
<td>5.1</td>
</tr>
<tr>
<td>% increase</td>
<td>11.11</td>
<td>1.1</td>
<td>21.38</td>
<td>19.9</td>
<td>22.3</td>
<td>0.79</td>
</tr>
<tr>
<td>HI 1011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>90.6</td>
<td>17.6</td>
<td>43</td>
<td>16.3</td>
<td>5.26</td>
</tr>
<tr>
<td>A</td>
<td>15.3</td>
<td>90</td>
<td>21.6</td>
<td>42.3</td>
<td>19.6</td>
<td>5.33</td>
</tr>
<tr>
<td>% increase</td>
<td>17.69</td>
<td>22.72</td>
<td>–</td>
<td>20.24</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>C306</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>14.3</td>
<td>109</td>
<td>19.6</td>
<td>21.6</td>
<td>10.3</td>
<td>5.3</td>
</tr>
<tr>
<td>A</td>
<td>17</td>
<td>109.3</td>
<td>21</td>
<td>24</td>
<td>11</td>
<td>5.43</td>
</tr>
<tr>
<td>% increase</td>
<td>18.88</td>
<td>0.27</td>
<td>7.14</td>
<td>11.11</td>
<td>6.79</td>
<td>2.45</td>
</tr>
</tbody>
</table>

observed in the rhizosphere of 6B, 1D, 7B and 5D. Also, these lines’ yield and nutrient content and uptake were affected significantly and positively.

Thus it can be stipulated that the genes/QTLs for response to inoculation of A. chroococcum for micronutrient uptake are present on 6B, 1D, 7B, 5D disomic chromosomes. Such genes/QTLs can be exploited to develop nutrient use efficient wheat varieties suitable for organic regimes and /or sustainable agriculture under integrated nutrient management.

Response of Wheat Genotypes to Azotobacter Inoculation under Rainfed Conditions

Approximately 26 percent of the total area under wheat cultivation in India is rainfed, where the crop grows largely on conserved soil moisture from preceding monsoon rains. Water deficits, poor soil fertility, low use of inorganic fertilizers, poor organic carbon and very poor rhizospheric activity in predominantly light textured soils are major factors in the low wheat production in such areas. Narula et al. (1998) conducted experiments under rainfed conditions to evaluate the responses of 10 wheat genotypes to Azotobacter inoculations. Significant wheat genotype x Azotobacter interaction suggested that some genotypes responded more to the bacterium than others. Only five genotypes recorded satisfactory grain yield (Table 2).

Drought tolerant genotype WH 533 significantly out yielded others, however, the control and treated plots were similar in yield. On the other hand, the heat tolerant mutant WH 147M and the drought tolerant genotype HI 1011 showed significant responses to Azotobacter compared to their respective controls. The genotypes, on average, manifested about an 18% increase in grain yield and spikelets/spikes. Likewise, WH 147M and HI 1011 showed about 9 and 17% increase, respectively, for tillers per plants. Variety C 306 had the maximum response to Azotobacter inoculation in tillers/plant, while PBW 175 had the maximum response in biological yield.

CONCLUSIONS

Under the present day energy crisis there is an urgent need to harness the potential benefits of bioinoculants like Azotobacter to sustainably enhance wheat production for food security. Yadav et al. (2000) evaluated various...
strains of *Azotobacter chroococcum*'s effect on wheat, particularly nitrogen fixation, ammonia excretion, production of siderophores, plant growth promoting substances and antimicrobial substances. Pot culture studies showed that yield attributes, such as plant height, biomass and grain yield, increased due to inoculation with *Azotobacter* strains, with and without added nitrogen. Several farmer field trials showed that strains Mac 27 and Ala 27 when applied with three-fourths of the recommended dose of nitrogen, increased yields to the same level obtained with a full recommended dose of nitrogen without any bacterial inoculation. Thus, a net saving of 30 kg of nitrogen can be achieved by inoculating with strains Mac 27 and Ala 27 and applying 90 kg N/ha. Approximately 92–95% of yield obtained with the full recommended dose of nitrogen were obtained using these strains with 60 kg N/ha. Hence, the use of *Azotobacter* as a bioinoculant has economic relevance which will increase further with the intensification of research and development.

The survival and competitive ability and performance of the improved bioinoculant strains must be improved for different agro-ecological niches (Bowen & Rovira, 1999). Appropriate management practices and compatible wheat genotypes need to be provided to ensure maximum contribution from bioinoculants (McSpadden Gardener & Fravel, 2002; Nelson, 2004). Host controlled factors play an important role in regulating the effects of bioinoculants, but, with the exception of a few cases, have not received their share of attention by researchers (Smith & Goodman, 1999; Mansouri, 2002). There is genotypic variation in the response to *Azotobacter* inoculation and the expression of this variability is greatest under low input conditions. Hence, it is possible to select and breed wheat genotypes under low input conditions. Plant genes/QTLs should be identified by monitoring the host response to bioinoculants. Pyramiding of such genes/QTLs is mostly available in traditional varieties, so local land races should be done, while also focusing on agronomically superior genotypes for high and low input systems. Research should be intensified to establish an effective associative/endophytic system in wheat by identifying effective bioinoculant strains and receptive wheat genotypes. Use of PCR based molecular markers should help unravel mechanisms involved in genotype-microbe interactions in specific agro-ecological conditions to harness favorable interactions for better understanding and application.

REFERENCES


Harnessing Wheat Genotype x Azotobacter Strain Interactions for Sustainable Wheat Production in Semi Arid Tropics


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