

Review

***Rhizobium* as a crop enhancer and biofertilizer for increased cereal production**

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Greater production of cereals brings forth higher production cost and pollutes the soil environment due to excessive use of chemical fertilizers. Therefore, crop scientists are exploring an alternative source namely biofertilizers which are cost effective and environment friendly. In the biofertilizer technology, *Rhizobium*-legume is most common and widely used in different countries. Recently, it is also found that rhizobia can make an association with graminaceous plants such as rice, wheat, maize, barley millets and other cereals some time as endophytic without forming any nodule-like structure or causing any disease symptoms. Increasing the ability of rhizobia in biofertilizer, crop enhancing activity in non-legumes especially cereal grains would be a useful technology for increased crop yields among resource-poor farmers. Recent findings showed both more crop enhancing and biofertilizer attributes in cereal crops due to rhizobial inoculation. In addition, plant nutrients like P, K, Ca, Mg and even Fe accumulation were also observed. Therefore, further research in this area will be able to develop a sustainable biofertilizer technology for greater and environment friendly cereal production system.

Key words: Biofertilizer, cereal, crop enhancer, *Rhizobium*, yield.

INTRODUCTION

The United Nations Food and Agriculture Organization (FAO) estimates that the total demands for agricultural products will be 60 percent higher in 2030 than present time. And more than 85% of this additional demand will come from developing countries. For over half a century, the world has relied on increasing crop yields to supply an ever increasing demand for food. World cereal production increased significantly during last two decades. This dramatic increase in world grain production was the result of a 122 percent increase in crop yields. However, this trend of grain production cannot be maintained due to decreasing cultivable land for rapid urbanization. Therefore, vertical expansion for food production is necessary. In order to increase world food production in a

sustainable manner, farmers have to use balanced fertilizer timely. And it is exciting that much of cereals especially rice is produced in countries where rapidly growing population, coupled to limited amounts of land and scares resources. One of the most important factors in the generation of high yields from modern rice cultivars is nitrogen fertilizer. That is why farmers are applying high amounts of the fertilizers which is very costly and make the environment hazardous especially when use discriminately. In addition, more than 50% of the applied N-fertilizers are some how lost through different processes which not only represent a cash loss to the farmers and consequently polluted the environmental (Ladha et al., 1998). Crop scientists all over the world are facing this alarming situation and they are trying to overcome this condition by exploring alternative sources which is cost effective and save the environment. Biofertilizer, an alternative source of N-fertilizer, especially rhizobia in legume symbiosis is an established technology. Use of the biofertilizers can also prevent the depletion of the soil organic matter (Jeyabal and Kuppaswamy, 2001). Inoculation with bacterial biofertilizer may reduce the applica-

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Abbreviations: BNF, Biological nitrogen fixation; LCO, lipochito-oligosaccharide; N₂, nitrogen; IAA, indole acetic acid; PGPB, plant growth promoting bacteria; GC-MS, gas chromatography- mass spectrometer.

tion of fertilizer-N by increasing N uptake by plants (Choudhury and Kennedy, 2004; Kennedy et al., 2004; Mia et al., 2005 and 2007). But most of this technique mainly limited between legume and *Rhizobium* in symbiotic process, which can fix atmospheric N₂. However, biological N₂ fixation (BNF) technology can play a vital role in substituting for commercially available N-fertilizer in cereal production thereby reducing the environmental problem to some extent. BNF and its transfer of NH₄ activate the growth promotion of associated plants. Nitrogen fixation and plant growth enhancement by rhizosphere bacteria might be important factors for achieving a sustainable agriculture in the future. This is associated with roots and grasses have been recognized as an important component of the nitrogen cycle in a range of ecosystems (Chalk, 1991). In this review, attempt has been made to summarize the current knowledge and information about rhizobial inoculation on cereal production. Information gathered through this review may yield new insights into recent development of rhizobia-cereal interaction.

NITROGEN IN CEREAL NUTRITION

Supply of nitrogen to crop plants especially cereals is critical for attaining yield potential, which is a highly demanding key element for poaceaeous plants as N cannot be stored in the roots. Nitrogen is a mobile element in the plant system, when the supply is suboptimal, plant growth is retarded, N mobilized from mature leaves and translocated to the new leaves and deficiency symptoms quickly develop in older leaves (Marschner, 1995). Cereal plants require large amounts of mineral nutrients including N for their growth, development and grain production. Rice crops remove around 16 - 17 kg N for the production of each tone of rough rice including straw (De Datta, 1981; Ponnampereuma and Detruck, 1993; Sahrawat, 2000). Nitrogen should be applied at short intervals during growth whereas other elements may be applied at planting. The relationship between total dry matter production and total N uptake is a close one, even taking into account the different varieties and varying environmental and soil conditions (Lahav, 1995). Cereals suffer from a mismatch of its N demand and it's supplied through chemical fertilizer, resulting in substantial loss of the fertilizer applied. Two approaches might be used in solving this problem namely regulating the timing of N application based on the plant needs while the other is to increase the efficiency of the use of available soil N and meet the additional N-demand by making cereal plants capable of "fixing its own nitrogen" through close association with diazotrophic bacteria. Achievement of the latter goal is a long-term strategy, but its potential has a considerable payoff in term of increasing cereal production, and helping resource-poor farmers as well as saving the environment (Cassman et al., 1997; Ladha et al., 1997).

Application of higher N may results in NO₃⁻ pollution of groundwater (Shrestha and Ladha, 1998), acidification of soil (Kennedy and Tchan, 1992) and increased denitrification resulting in higher emission of N₂O to the atmosphere, which may impact global warming (Bronson et al., 1997). In addition, the burning of fossil fuel for manufacturing N-fertilizers is a source of hazardous byproducts that pose a threat to human health and the environment (Vitousek et al., 1997). These problems have made wariness-replenished public interest in exploring alternate or supplementary nonpolluting sources of N for sustainable agriculture (Ladha et al., 1997).

Biofertilizers, microbial inoculants that can promote plant growth and productivity, are internationally accepted as an alternative source of N-fertilizer. They are environmentally friendly and can be used to ensure a sustainable cereal production. In the biofertilizer technology, new systems are being developed to increase the biological N₂ fixation (BNF) with cereals and other non-legumes by establishing N₂-fixing bacteria within the roots (Cocking, 2000). Nitrogen fixation and plant growth promotion by rhizobacteria are important criteria for an effective biofertilizer.

RHIZOBIUM

Rhizobia are soil bacteria that fix N₂ (diazotroph) after becoming established inside root nodules of legumes (Fabaceae). There are several different genera of rhizobia, all of them belong to the Rhizobiales, a probably-monophyletic group of proteobacteria and they are soil bacteria characterized by their unique ability to infect root hairs of legumes and induce effective N₂-fixing nodules to form on the roots. They are rod shaped living plants which exist only in the vegetative stage. Unlike many other soil microorganisms, rhizobia produce no spores and they are aerobic and motile. Rhizobia (species of *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium* and *Sinorhizobium*) form intimate symbiotic relationships with legumes by responding chemotactically to flavonoid molecules released as signals by the legume host. These plant compounds induce the expression of nodulation (nod) genes in rhizobia, which in turn produce lipo-chito-oligosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (Matiru and Dakora, 2004; Dakora, 1995; Lhuissier et al., 2001).

The legume-*Rhizobium* symbiosis is a typical example of mutualism, but its evolutionary persistence is actually somewhat surprising. Because several unrelated strains infect each individual plant, any one strain could redirect resources from N₂ fixation to its own reproduction without killing the host plant upon which they all depend. It turns out that legume plants guide the evolution of rhizobia towards greater mutualism by reducing the oxygen supply to nodules that fix less N₂ thereby reducing the

frequency of cheaters in the next generation. Symbiotic N₂-fixation has been studied widely and exploited as a means of increasing crop yields (Boholool, 1990; Sharma et al., 1993). The fixed N₂ is released when the plants die, making it available to other plants and this helps in fertilizing the soil. The great majority of legumes have this association, but a few genera (e.g., *Styphnolobium*) do not. Photosynthetic *Bradyrhizobium* were found as natural endophytes of the African wild rice *Oryza breviligulata* (Chaintreuil et al., 2000).

AGROBACTERIUM: A RHIZOBIA RELATED BACTERIA

Agrobacterium, genera of *Rhizobiaceae* family, is an artificial genus comprising plant-pathogenic species, and based on the DNA analysis, Young et al. (2001) proposed to rename *Agrobacterium rhizogens* and *Agrobacterium radiobacter* as *Rhizobium rhizogens* and *Rhizobium radiobacter*. Although *Agrobacterium* spp. have been isolated from the nodules of several tropical legumes but none of these have been shown to be capable of nodulating the host plant (de Lajudie et al., 1999; Mhamdi et al., 2005). Conversely, Kanvinde and Sastry (1990) suggested that *A. tumefaciens* strains B6, C58 and NT₁ can fix atmospheric N₂. Nevertheless, recently, the coexistence of symbiosis and pathogenicity-determining genes has been shown to occur in *Rhizobium rhizogenes* a strain which enables them to induce nodules or tumors depending on plant species (Vela'zquez et al., 2005). *A. rhizogens*, free living N₂ fixing bacteria, are capable of inducing a hairy-root (rhizogenic) reaction in host plants (Conn, 1942). *A. radiobacter* described as non-pathogenic *Agrobacterium* strains, which are capable of N₂ fixation and IAA production. Inoculation of wheat and barley with a bacterial strain isolated from the rhizosphere of a rice crop growing in the USSR and initially identified as *A. radiobacter*, resulted in crop yield increases equivalent to that obtained by the application of 30 kg N ha⁻¹ (Bairamov et al., 2001; Zavalin et al., 2001).

COLONIZATION OF RHIZOBIA IN THE ROOTS OF CEREAL CROPS

These beneficial effects require a successful colonization of rhizobia on the roots of the host plant to create a conducive environment for increased interaction between bacteria and the roots. The attachment of rhizobia to the root is essential for the establishment of an efficient association with the plants. *Rhizobium* is a root colonizer, plant specific, thus cannot colonize a wide range of plant species. Although rhizobia naturally infect legumes as host plants, some *Rhizobium* strains can form symbiotic relationships with non-legumes species such as *Parasponia* (Trinick, 1979). Infection and colonization of a

non-legume by the N₂ fixing bacteria differ significantly from *Rhizobium*-legume symbiosis. But rhizobial colonization in non-legumes extends from rhizosphere into the epidermis, endodermis and cortex, but the main site of colonization is intercellular space of rice roots (Reddy et al., 1997). Using various techniques like fluorescent-tagged endophytes, antibodies, fluorescent microscopy, scanning and transmission electron microscopy, the bacterial route of entry into the host plant has been traced and scored in many cases (Prayitno et al., 1999; Chaintreuil et al., 2000; Verma et al., 2004; Perrine-Walker et al., 2007). Sometimes endophytes are a more aggressive colonizer and are capable of out-competing others in surroundings (Verma et al., 2004). Some kind of quorum-sensing mechanism as in *Rhizobium*-legume symbiosis might also exist in this case which helps in the establishment of successful relationship. Rhizobial inoculation to cereal plants especially rice is associated with an increased accumulation of phenolic substances such as gallic, tannic, ferulic and cinnamic acids in plants leaves (Mirza et al., 2001). Such increases in phenolic acids are a pathogenic stress related phenomenon in plants (Pieterse et al., 2002). Defense reactions triggered in response to rhizobial invasions is termed as rhizobacteria mediated induced systemic resistance. After a successful infiltration, *Rhizobium* is disseminated throughout the host plant interior without evoking an observable defense reaction in the plant. Similarly, *Azorhizobium caulinodans* were able to enter the roots of rice at emerging lateral roots (lateral root cracks) by crack entry and bacteria moved into intercellular space within the cortical cell layer of roots. Lateral root crack colonization of rice was also observed with similar frequency following inoculation with *Azospirillum brasilense* and the colonization by *A. brasilense* was stimulated by naringenin and other flavonoid molecules (Jain and Gupta, 2003). Some strains may have the ability to infect rice root tissue via root hairs located at the emerging lateral roots and to spread extensively throughout the rice root (Francine et al., 2007; Gough et al., 1996; Ladha et al., 1996). Some naturally occurring rhizobia can invade the emerging lateral roots of rice, wheat, maize and oilseed rapes (Cocking et al., 1990, 1992, 1994). Bacteria also invade the host plant root system by crack entry infection that is intercellularly between adjacent plant cells and not by the formation of infection threads and the tip of root hairs (Sprent and Raven, 1992).

FORMATION OF NODULE IN DIFFERENT NON-LEGUMES

An important requirement for efficient biological BNF is to have diazotrophic bacteria growing endophytically within plants, as in legume-rhizobia (van Rhijn and Vanderlyden, 1995), *Parasponia-Bradyrhizobium* (Webster et al., 1995) and *Actinorhiza-Frankia* (Benson and Silvester, 1993) symbiosis. In these associations,

specialized organs called nodules are formed in which bacteria reduce N_2 into NH_4 and this fixed- N_2 is transformed to plants (Jain and Gupta, 2003). During the last few years, there has been an increased interest in exploring the possibilities of extending the beneficial interactions between cereals with plant growth promoting bacteria on the potential for nodulation and N_2 fixation in cereal with symbiotic process (Khush and Bennett, 1992). Rhizobia are able to induce, at low frequency, nodule-like structures on the roots of rice, wheat and oilseeds rape seedlings. Nodular structures have been induced on rice roots by treating the roots of 2-day-old seedlings with a cell wall degrading enzyme mixture consisting of 1.0% cellulase YC, 0.1% pectolyase Y23 and 8% mannitol, followed by inoculation with rhizobia in the presence of polyethylene glycol. Rhizobia were located both within the degenerating cytoplasm of cells and between the cells of spherical and elongated nodular structures. Although nitrogenase activity in such structures was at the limit of sensitivity of the assay procedure, this first report of the induction of nodular structures on rice by rhizobia opens up the possibility of extending effective nodulation to non-legumes, including cereals by facilitating the entry of rhizobia through cell wall degradation (Al-Mallah et al., 1989, 1990).

The effect of flavonoids on bacterial colonization of wheat and rice roots was also investigated and demonstrated that flavonoid and naringenin is able to stimulate *Azorhizobium* colonization of non-legume root systems (Gough et al., 1996). Effective nodulation has also been observed in *Parasponia andersonii* following *Bradyrhizobium* inoculation of plantlets regenerated from chilli (Davey et al., 1993).

CROP ENHANCING ACTIVITY

Root stimulation for water and nutrient uptake

It is well-known that inoculation of cereals with plant growth promoting bacteria (PGPB) resulted in increased plant/crop growth and yield and acts as biofertilizer and bioenhancer for different non-legumes (Andrews et al., 2003; Mia et al., 2009; Shamsuddin et al., 1998). However, there is general agreement that these growth responses were not due to N_2 fixation by the bacterium, but were primarily related to the bacterial production of phytohormone, which caused changes in root morphology and physiology that resulted in increased nutrient and water uptake from the soil (Andrews et al. 2003; James, 2000; Mantelin and Touraine, 2004; Mia et al., 2009). Similar crop enhancing activity in different non-legumes has been observed due to rhizobial inoculation. These growth enhancing phenomena are more pronounced in roots rather shoot. Roots in terrestrial plants serve various functions: conduction or supply of both nutrients and water to the plants from the soil, a location for the synthesis and exchange of various plant hormones, storage organs of plant resources and

the anchorage of the plant (Fitter, 1991; Schiefelbein and Benfey, 1991). Root development is clearly influenced by the phytohormone auxins, which are known to influence a number of plant functions such as promotion of cell elongation and cell division, apical dominance, root initiation, differentiation of vascular tissue, ethylene biosynthesis mediation of tropic responses, and alteration of the expression of specific gene (Chasan, 1993; Key, 1989; Sachs, 1993; Warren-Wilson and Warren-Wilson, 1993). There are evidences that certain strains of *Rhizobium* can enhance rice growth and yield through the accompanying changes in growth physiology or root morphology rather than biological N_2 fixation (Biswas et al., 2000). *Rhizobium* spp. are capable of synthesizing IAA in absence of tryptophan, but the exogenous application of tryptophan increases IAA production several fold (Kittell et al., 1989). Increased rice root systems with a significantly greater absorptive surface area and extra cellular bioactive metabolites that can promote rice root development resulting in expansive root architecture were also observed by the application of *Rhizobium*.

Recently, Yanni et al. (1997) and Biswas (1998) showed increased N uptake by rice plants inoculated with rhizobia, which raises questions of whether this benefit of rhizobia to rice may be due to their associative N_2 fixing activity and/or their ability to change the phytohormone balance, thereby influencing growth physiology in ways that affect major nutrient uptake in rice.

Promotion of shoot growth

Rhizobium spp. are plant growth promoting rhizobacteria and some are endophytes which can produce phytohormones, siderophores, HCN, solubilize sparingly soluble organic and inorganic phosphates and can colonize in the roots of many non-legumes (Antoun et al., 1998; Sessitsch et al., 2002). With the use of high-performance liquid chromatography or GC-MS, auxin synthesis by *Rhizobium* spp. has now been unequivocally demonstrated by researchers (Sekine et al., 1988). Growth promotion of rice cultivars Giza-177, Sakha-102, L204 (*Indica*) and M202 (*Japonica*) were observed by the inoculation of *Rhizobium*. The association increased plant growth at different growth stages such as enhanced seed germination, increased shoot length, leaf chlorophyll content, total dry matter, grain yield, N content and yield attributes (Biswas et al., 2000a; Prayitno et al., 1999; Yanni et al., 1997). Biswas et al. (2000b) reported that enhanced vigor at seedling stage resulted in a carryover effect of significantly increased straw and grain yields at maturity stage when the culturable population of the inoculants lessen below detectable levels.

BIOFERTILIZER ACTIVITY AND YIELD INCREMENT

It has been found from various experimental results that inoculation of *Rhizobium* in different cereal grains

Table 1. Yield improvement and other beneficial effects of *Rhizobium* with cereal grains.

Host plants	<i>Rhizobia</i>	Colonization	Growing condition	Percent increase	References
Rice	<i>Bradyrhizobium</i>	Rhizosphere	Gnotobiotic	20 (total biomass)	Chaintruel et al. (2000); Bhattacharjee et al. (2008).
Rice	<i>Rhizobium leguminosarum</i> bv.trifolii <i>R. vietnamiensis</i>	Roots Rhizosphere	Greenhouse and field Nursery pot trial, field pot trial	15 - 22, 8 - 22 (grain yield) 23 and 59 (shoot/root weight), 19 (yield), 13 - 22 (yield)	Yanni et al. (1997, 2001). Biswas et al. (2000a,b) . Tran Van et al. (2000).
Rice	<i>R. leguminosarum</i>	Rhizosphere	Pot culture Glasshouse	43 (yield) 18 (biomass) 25 (grain size)	Hussain et al. (2009).
Wheat	<i>R. trifolii</i>	roots	Pot trials	24 (wheat shoot dry matter and grain yield)	Hilali et al. (2001).
Maize	<i>R. trifolii</i>	roots	Greenhouse, field	34 (yield), 11 (yield)	Riggs et al. (2001).
Maize	<i>Sinorhizobium</i> spp.		Green house	49 - 82 (yield)	Riggs et al. (2001).
Maize	<i>R. etli</i> bv. <i>phaseoli</i>	roots	Gnotobiotic	20 - 45 (total biomass)	Zamora and Matinez-Romero (2001).

increased yield to some extent. Table 1 summarized some of the findings showing increments of different yield attributes. Yanni et al. (1997) and Biswas (1998) reported increased N uptake by rice plants inoculated with rhizobia. This plant response is significant because of its potential importance to sustainable agriculture, especially in cropping systems involving rotations of rice and legumes. It raises questions of whether this benefit of rhizobia to rice may be due to their associative N₂ fixing activity and/or their ability to change the phytohormone balance, thereby influencing growth physiology in ways that affect major nutrient uptake in rice (Biswas et al., 2000). It was observed that rhizobial inoculation enhanced stomatal conductance, thereby increasing the photosynthesis rates by 12% in rice varieties

where 16% grain yield was recorded. A positive correlation between increased grain yield and photosynthetic rate at zero N-level was also found (Peng et al., 2002). Similarly, Mia et al. (2000) found increased photosynthetic rate and yield in Bananas inoculated with PGPR. They also found high and quality banana produced by inoculation (Mia et al., 2005). A general decrease in performance was observed when the pot grown inoculated plants are shifted to the field (Riggs et al., 2001; Gyaneshwar et al., 2002). Some of the factors that may affect the performance of inoculums are soil type, organic matter and other soil physical factors. High concentration of N-fertilizers especially NH₄NO₃ change the morphology and activity of N₂ fixing bacteria consequently causing harmful effects (Muthukumarasamy et al.,

2002). Yield and nodulation were found to have significant positive correlation for both crop seasons. For the bean-sorghum intercropped conditions, the use of mixed granular rhizobial inoculant and starter nitrogen fertilizer is indispensable to realize the benefits of BNF. Sorghum in the *kharif* season (July – October) followed by chickpea in *rabi* season (November – February) is an important crop rotation under semi-arid regions. Integrated nutrient management including the use of a combination of inorganic, organic and bio-fertilizers for enhancing crop growth and sustaining yields holds great promise for farmers. Biswas et al. (2000) reported that inoculation with *Rhizobium leguminosarum* bv. *trifolii* E11, *Rhizobium* sp. IRBG74 and *Bradyrhizobium* sp. IRBG271 increased rice grain and straw yields by

8 to 22 and 4 to 19%, respectively, at different N rates. Nitrogen, P and K uptake were increased by 10 to 28% due to rhizobial inoculation which also increased Fe uptake in rice by 15 to 64%. It is suggested that promoting effects of *Rhizobium* and *Bradyrhizobium* inoculation should be considered not only as symbiotic N₂-fixers for legumes but also as PGPR producers for legume and non-legume to increase shoots and yield under drought stress. This increase might be attributed to changes in many metabolic and physiological processes. Finally, cell viability and electrolyte leakage tests as well as chemical constituents can be used to select drought tolerant cultivars (Rashad et al., 2001). In the field experiment in rice, *R. leguminosarum* inoculation increased in biomass at vegetative stage in Vietnam condition (Kennedy et al., 2004).

BIOCONTROL ACTIVITY

Certain mechanisms are postulated towards *Rhizobium* which may be involved in growth promoting activities, that is, mobilization and efficient uptake of nutrient, enhancement of stress tolerance, solubilization of insoluble phosphate, induction of systematic disease resistance, production of phytohormones, vitamins and siderophores (Biswas et al., 2000; Mayak et al., 2004; Alikhani et al., 2006; Tuzun and Kloepper, 1994; Dakora, 2003; Dobbelaar et al., 2003; Neil and Leong, 1986). *Rhizobium* has potential use as biocontrol agents and Ehteshamul-Haque and Ghaffer (1993) found that *S. melliloti*, *R. leguminosarum* bv. *viciae* and *B. japonicum* reduced infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. in both leguminous and non-leguminous plants. Bacterial biological control agents have been successfully developed for other *Rhizobium* spp. (Burr and Reid, 1994). In replicated field trials, *Rhizobium radiobacter* strain K84 has provided effective field control against tumorigenic forms of *R. radiobacter* that affect stone fruits and many other tree crops (Schroth and Moller, 1976). One of the factors contributing to the success of this biocontrol agent is its persistence in the rhizosphere where control is required. The persistence of potential biological control bacteria in the xylem is crucial for activity against the bacteria that cause crown gall disease.

FUTURE PROJECTION AND CHALLENGES

Recent advances in knowledge of symbiotic *Rhizobium*-legume association at the molecular level and the ability of incorporating new genes into cereals through transformation have created excellent prospect to investigate the possibilities for integrating N₂ fixing capability in non-legumes especially in rice (Ladha et al., 1997). Now the challenge is to establish stable *Rhizobium*-cereal associations and major emphasis should be given for efficient

and stable associations including endophytic association. Conditions which give reproducible invasion of lateral root cracks and subsequent intercellular colonization of the roots of rice and wheat by rhizobia at high frequency, provides important information to researchers attempting to extend rhizobial colonization and endophytic N₂ fixation to non-legume crops (Webster et al., 1997). Liquid inocula of *Rhizobium* should be developed for easy handling and foliar spray. Consistency in achieving yield and grain quality will be a big challenge in this technology.

CONCLUSIONS

Information from the various literatures available presently depicts that association either rhizospheric or endophytic between *Rhizobium* and cereal is a natural phenomenon. Progressive knowledge of this area may bring benefits for using this technology. For commercialization, extensive optimization and comparative study of the after effects of the application is required. Hence, more research is needed on the interaction between cereal grains and rhizobia or rhizobia-like bacteria. There is also need to fully assess whether the rhizobia fix N₂ in association with cereals under conditions in which this bacteria promote plant growth and if so what portion of the plant-N can be derived from BNF process. Our understanding of the natural ability of rhizobia to inhibit cereals and enhance their growth is only beginning to be explored. Nevertheless, the novel findings described here represent major steps forward in achieving the technically challenging goal of increasing cereal productivity by reducing its dependence on the chemical fertilizer-N through enhancement of its natural association with rhizobia.

REFERENCES

- Alikhani HA, Saleh-Rastin, N Antoun H (2006). Phosphate solubilization activity of rhizobia native to Iranian soils. *Plant Soil*, 287:35-41.
- Al-Malah MK, Davey MR, Cocking EC (1989). Formation of nodular structures on rice seedlings by rhizobia. *J. Exp. Bot.* 40:473-478.
- Al-Mallah MK, Davey MR, Cocking EC (1990). Nodulation of oilseed rape (*Brassica napus*) by rhizobia. *J. Exp. Bot.* 41: 1567-1572.
- Andrews M, James EK, Cummings SP, Zavalin AA, Vinogradova LV, McKenzie BA (2003). Use of nitrogen fixing bacteria inoculants as a substitute for nitrogen fertilizer for dry land graminaceous crops: progress made mechanisms of action and future potential. *Symbiosis* 35:209-229.
- Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalandi R (1998). Potential of *Rhizobium* and *Bradyrhizobium* as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant Soil*. 204:57-68.
- Bairamov LE, Vinogradova LV, Zavalin AA (2001). Nitrogen nutrition and productivity of barley as conditioned by the application of associative diazotrophs. *Appl. Biol.* 63:135-139.
- Benson DR, Silvester WB (1993). Biology of *Frankia* strains, actinomycete symbionts of actinorhiza plants. *Microbiol. Mol. Biol. Rev.* 57(2): 293-319.
- Bhattacharjee RB, Singh A, Mukhopadhyay (2008). Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: Prospects and challenges. *Appl. Microbiol Biotechnol.* 80: 199-209.

- Biswas JC (1998). Effect of nitrogen fixing bacteria on growth promotion of lowland rice (*Oryza sativa* L.). Ph.D. thesis. Dept. of Soil Sci., Univ. of Philippines, Los Banos.
- Biswas JC, Ladha J K, Dazzo FB (2000a). Rhizobia inoculation improves nutrient uptake and growth of lowland rice. *Soil Sci. Soc. Amer. J.* 64:1644-1650.
- Biswas JC, Ladha J K, Dazzo FB, Yanni YG, Rolfe BG (2000b). Rhizobial inoculation influences seedling vigor and yield of rice. *Agronomy J.* 92:880-886.
- Boholool B (1990). Introduction to nitrogen fixation in agriculture and industry: Contribution of BNF to sustainability of agriculture. pp. 613-616 *In: Chalk PM, Gresshoff MM, Roth LE, Stacey G, Newton WE (ed) Nitrogen Fixation: Achievements and Objectives*, Chapman and Hall, New York.
- Bronson KF, Singh U, Neu HU, Abao JEB (1997). Automated chamber measurements of methane and nitrous oxide flux in a flooded rice soil: Fallow period emissions. *Soil Sci. Soc. Amer. J.* 61:988-993.
- Burr JJ, Reid CL (1994). Biological control of grape crown gall with non-tumorigenic *Agrobacterium vitis* strain Fe/5. *Amer. J. Enol. Vitric.* 45: 213-219.
- Cassman K G, Peng S, Olk D C, Ladha J K, Rechartd W, Doberman A, Singh U (1997). Opportunities for increased nitrogen use efficiency from improved resource management in irrigated rice systems. *Field Crops Res.* 56, pp. 7-39.
- Chasan R (1993). Embryogenesis: new molecular insights. *The Plant Cell* 5: 597-599.
- Chaintreuil C, Giraud E, Prin Y, Lorquin J, Ba A, Gillis M, de Lajudie P, Drefus B (2000). Photosynthetic Bradyrhizobia are natural endophytes of the African wild rice *Oryza breviligulata*. *Appl. Environ. Microbiol.* 66:5437-5447.
- Chalk P M (1991). The contribution of associative and symbiotic nitrogen fixation to the nitrogen nutrition of non-legumes. *Plant Soil* 132:29-39.
- Cocking EC, Al-Mallah MK, Benson E, Davey MR (1990). Nodulation of non-legumes by rhizobia. *In: Nitrogen Fixation Achievements and Objectives*. Eds. Gresshoff PM, Roth EC, Stacey G, Newton WE. pp. 813-823. Chapman and Hall. New York, USA.
- Cocking EC, Davey MR, Kothari SL, Srivasta JS, Jing Y, Ridge RW, Rolfe BG (1992). Altering the specificity control of the interaction between rhizobia and plants. *Symbiosis*. 14:123-130.
- Cocking E C, Webser G, Batchelor CA, Davey, MR(1994). Nodulation of non-legume crops. A new look. *Agro-Food-Industry Hi-Tech, January/February*, pp. 21-24.
- Cocking E C (2000). Helping plants get more nitrogen from air. *European Review* 8(2):193-200.
- Choudhury ATMA, Kennedy IR (2004). Prospects and potentials for system of biological nitrogen fixation in sustainable rice production. *Biol. Fertil. Soils.* 39:219-227.
- Conn HJ (1942). Validity of the genus *Alcaligenes*. *J. Bact.* 44:353-360.
- Davey MR, Webster G, Manders G, Ringrose FL, Power JB, Cocking EC (1993). Effective nodulation of micro-propagated shoots of the non-legume *Parasponia andersonii* by *Bradyrhizobium*. *J. Exp. Bot.* 44: 863-867.
- Dakora FD (1995). Plant flavonoids: biological molecules for useful exploitation. *Aust. J. Plant Physiol.* 22:7-99.
- Dakora FD (2003). Defining new roles for plant and rhizobia molecules in sole and mixed plant cultures involving symbiotic legumes. *New Phytol.* 158:39-49.
- De Datta SK (1981). Principles and practices of rice production. John Wiley & Sons, New York.
- de Lajudie P, Willems A, Nick G, Mohamed SH, Torck U, Coopman R, Filali-Maltouf A, Kersters K, Dreyfus B, Lindstrom K, Gillis M (1999). *Agrobacterium* bv. 1 strain isolated from nodules of tropical legumes. *Syst. Appl. Microbiol.* 22:119-132.
- Dobbelara S, Vanderleyden J, Okon Y. (2003). Plant growth promoting effects of diazotrophs in the rhizosphere. *Plant Soil* 22:107-149.
- Ehteshamul Haque SRY, Gaffar A. (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mung-bean. *J. Phyt.* 138:157-163.
- Fitter AH (1991). Characteristics and functions of root system in plant roots: The Hidden Half. eds.: Waisel Y, Eshel A Kafkafi U. pp. 3-25. Marcel Dekker, Inc, New York.
- Francine M, Walker P, Prayitn J, Rolfe BG, Weinman JJ, Hocar HC (2007). Infection process and the interaction of rice roots with rhizobia. *J. Exp. Bot.* 58(12):3343-3350.
- Gough C, Webster G, Vasse J, Galera C, Batchelor C, O'Callaghan K, Davey M, Kothari S, Denerie J, Cocking E (1996). Specific flavonoids stimulate intercellular colonization of nonlegumes by *Azorhizobium caulinodans*. *In Biology of plant microb interactions*. eds. Stacey G, Mullin B, Gresshoff PM. Intl. Soc. Mol. Plant-Microb Interactions. Minnesota. pp. 409-415.
- Gyaneshwar P, James EK, Reddy PM, Ladha J (2002). *Herbaspirillum* colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. *New Phytol.* 154:131-145.
- Gutierrz-Zamora ML, Martinez-Romero E (2001). Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.). *J Biotechnol.* 91:117-126.
- Hilali A, Prevost D, Broughton WJ, Antoun H (2001). Effects de inoculation avec des souces de *Rhizobium leguminosarum* biovar *trifolii* sur la crossance du dans deux sols du Maroc. *Can. J. Microbiol.* 47 :590-599.
- Hussain MB, Mehboob I, Zahir ZA, Naveed M, Asghar HN (2009). Potential of *Rhizobium* spp. for improving growth and yield of rice (*Oryza sativa* L.). *Soil Environ.* 28(1): 49-55.
- Jain V, Gupta K (2003). The flavonoid naringen enhances intercellular colonization of rice roots by *Azorhizobium caulinodans*. *Biol. Fertil Soils.* 38:119-123.
- James EK (2000). Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res.* 65: 197-209.
- Jeyabal A, Kupuswamy G (2001). Recycling of organic wastes for the production of vermicompost and its response in rice-legume cropping system and soil fertility. *Eur. J. Agron.* 15:153-170.
- Kanvinde L Sastry GRK (1990). *Agrobacterium tumefaciens* is a diazotrophic bacterium. *Appl. Environ. Microbiol.* 56:2087-2092.
- Kennedy IR, Tchan Y (1992). Biological nitrogen fixation in nonlegumes filed crops: recent advances. *Plant Soil* 141:93-118.
- Kennedy IR, Choudhury ATMA, Kecskés ML (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biol. Biochem.* 36:1229-1244.
- Key JL (1989). Modulation of gene expression by auxin. *Bio-Essays* 11:52-57.
- Kittell BL, Helinski D R, Ditta G S (1989). Aromatic aminotransferase activity and indoleacetic acid production in *Rhizobium meliloti*. *J. Bacteriol.* 171: 5458-5466.
- Khush GS, Bennet J (1992). Nodulation and nitrogen fixation rice: Potential and prospect: International Rice Research Institute Press, Manila, Philippines. p.136.
- Ladha JK, Kundu DK, Coppenolle MGA, Peoples MB, Carangal VR, Dart PJ (1996). Legume productivity and soil nitrogen dynamics in lowland rice-based cropping systems. *Soil Sci. Soc. Amer. J.* 60:183-192.
- Ladha JK, Padre AT, Punzalan G C, Castillo E, Singh U, Reddy C K (1998). Nondestructive estimation of shoot nitrogen in different rice genotypes. *Agron. J.* 90:33-40.
- Ladha J K, de Bruijin F J, Malik K A (1997). Assessing opportunities for nitrogen fixation in rice: a frontier project. *Plant Soil* 194: 1-10.
- Lahav E (1995). Banana nutrition. *In: Bananas and Plantains*. ed: Gowen, S. R. pp. 258-316. Chapman & Hall, London.
- Lhuissier FGP, de Rujiter NCA, Sieberer BJ, Esseling JJ, Emons AMC (2001). Time of cell biological events evoked in root hairs by *Rhizobium* nod factors: state of the art. *Ann. Bot.* 87:289-302.
- Mantelin J, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *J. Exp. Bot.* 55:27-34.
- Marschner H (1995). Functions of mineral nutrients: Macronutrients in mineral nutrition of higher plants. pp. 889. Academic Press, London.
- Matiru VN, Dakora FD (2004). Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. *Afr. J. Biotechnol.* 3(1):1-7.
- Mayak S, tirosh T, Gilick BR (2004). Plant growth promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42:565-572.
- Mhamdi R, Mrabet M, Laguerre G, Tiwari R, Aouani ME (2005). Colonization of *Phaseolus vulgaris* nodules by *Agrobacterium*-like

- strains. *Can J Microbiol* 51:105– 111.
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2000). Growth and physiological attributes of hydroponically-grown bananas inoculated with plant growth promoting rhizobacteria. *Transac. Malaysian Soc. Plant Physiol.* 9: 324-327.
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2005). High-yielding and quality banana production through plant growth promoting rhizobacterial inoculation. *Fruits*: 60:179-185.
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2007). Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. *Infomusa* 16 (1&2): 11-15.
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2009). The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. *Afr. J. Biotechnol.* (Accepted).
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2009). Rhizobacteria as bioenhancer for growth and yield of banana (*Musa* spp. cv. "Berangan"). *Scientae Hort.* (Accepted).
- Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA (2001). Isolation, partial characterization, and effect of plant growth promoting bacteria (PGPB) on micropropagated sugarcane *in vitro*. *Plant Soil* 237:47-54.
- Muthukumarasamy R, Revathi G, Loganathan P (2002). Effect of inorganic N on the population, *In vitro* colonization and morphology of *Acetobacter diazotrophicus* (syn *Gluconacetobacter diazotrophicus*). *Plant Soil* 243:91-102.
- Neilands JB, Leong SA (1986). Siderophores in relation to plant growth and disease. *Ann. Rev. Plant Physiol.* 37:187–208.
- Peng S, Biswas JC, Ladha JK, Gyaneshwar P, Chen Y (2002). Influence of rhizobial inoculation on photosynthesis and grain yield of rice. *Agron. J.* 94:925-929.
- Pieterse CMJ, Van Wees SCM, Ton J, Van Pelt JA, van Loon LC (2002). Signaling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana* Plant Biol (Stuttgart). 4:535-544.
- Perrine-Walker FM, Gartner E, Hocart CH, Becker A, Rolfe BG (2007). *Rhizobium*-initiated rice growth inhibition caused by nitric oxide accumulation. *Molecular Plant-Microbe Interactions* 20:283-292.
- Ponnamperuma FN, Deturk P (1993). A review of fertilization in rice production. *Int. Rice Commun News* 42:1-12.
- Prayitno J, Stefaniak J, Mcleaver J, Weinmen JJ, Dazzo FB, Ladha JK, Barraquio W, Yanni YG, Rolfe BG (1999). Interactions of rice seedlings with bacteria isolated from rice roots. *Aust. J. Plant Physiol.* 26:521-535.
- Rashad MH, Ragab AA, Salem SM (2001). The influence of some *Bradyrhizobium* and *Rhizobium* strains as plant growth promoting rhizobacteria on the growth and yield of sorghum (*Sorghum bicolor* L.) plants under drought stress. Eds: Horst W J, Schenk M K, Bürkert A, Claassen N, Flessa H, Frommer W B, Goldbach H, Olf H W, Römheld V, Sattelmacher B, Schmidhalter U, Schubert S, Wirén N and Wittenmayer L. Plant nutrition-food security and sustainability of agro-ecosystems. pp. 664-665.
- Reddy PM, Ladha JK, So RB, Hernadez Ramos MC, Angeles OR, Dazzo FB de Bruijn, FJ (1997). Rhizobial communication with rice roots: induction of phenotypic changes, mode of invasion and extend of colonization. *Plant Soil* 194(1-2):81-98.
- Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW (2001). Enhanced maize productivity by inoculation with diazotrophic bacteria. *Aust. J. Plant Physiol.* 28:829-836.
- Sachs T (1993). The role of auxin in the polar organization of apical meristems. *Aust. J. Plant Physiol.* 20:541-553.
- Sahrawat KL (2000). Macro and micronutrients removed by upland and low land rice cultivars in West Africa. *Commun. Soil Sci. Plant Anal.* 31:717-723.
- Schrorth MU, Moller WJ (1976). Crown gall controlled in the field with a nonpathogenic bacterium. *Plant Dis. Rep.* 60:275-278.
- Schiefelbein JW and Benfey PN (1991). The development of plant roots: New approach to underground problems. *The Plant Cell* 3:1147-1154.
- Sekine M, Ichikawa T, Kuga N, Kobayashi M, Sakurai A, Syono K (1988). Detection of the IAA biosynthetic pathway from Tryptophan via indole-3-acetamide in *Bradyrhizobium* spp. *Plant Cell Physiol.* 29:867-874.
- Sessitsch A, Howieson JG, Perret X, Antoun H, Martínez-Romero E (2002). Advances in *Rhizobium* research. *Crit. Rev. Plant Sci.* 21: 323-387.
- Sharma PK, Kundu BS, Dogra RC (1993). Molecular mechanism of host specificity in legume-*Rhizobium* symbiosis. *Biotechnol. Adv.* 11:714-779.
- Sheresta RK Ladha JK (1998). Nitrate in groundwater and integration of nitrogen-catch crop in rice-sweet pepper cropping system. *Soil Sci. Soc Amer. J.* 62:1610-1619.
- Sprent JI, Raven JA (1992). Evolution of nitrogen fixing symbiotics. In: Biological nitrogen fixation. eds. Stacey G, Burn RH, Evans JH. pp. 461-496. Chapman and Hill, New York, USA.
- Tran Van V, Berge O, Ke SN, Balandreau J, Heulin T (2000). Repeated beneficial effects of rice inoculation with a strain of *Burkholderia vietnamiensis* on early and late yield components in low fertility sulphate acid soils of Vietnam. *Plant Soil* 218:273-284.
- Tuzun S, Kloepper J W (1994). Induced systemic resistance by plant growth-promoting rhizobacteria. In: Improving Plant Productivity with Rhizosphere Bacteria. Ryder M H, Stephens PM, Bowen G D, eds. CSIRO, Adelaide, South Australia, Australia. pp. 104-109.
- Trinick MJ (1979). Structures of nitrogen fixing root nodules formed on *Parasponia andersonii* (Planch). *Can. J. Microbiol.* 25:565-578.
- Van Rhijn P, Vandelyden J (1995). The *Rhizobium*-plant symbiosis. *Microbiol Review* 59: 124-142.
- Verma SC, Singh A, Chowdhury S, Tripathi AK (2004). Endophytic colonization ability of two deepwater rice endophytes, *Pantoea* sp. and *Ochrobacterium* sp. using green fluorescent protein reporter. *Biotechnol. Lett.* 26:425-429.
- Vela'zquez E, Peix A, Zurdo Pin'eiro JL, Palomo JL, Mateos PF, Rivas R, Mun'oz-Adelantado E, Toro N, Garcia-Benavides P, Martizez-Molina E (2005). The coexistence of symbiosis and pathogenicity-determining genes in *Rhizobium rhizogenes* strains enables them to induce nodules and tumors or hairy roots in plants. *MPMI* 18:1325–1332.
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.* 7:737–750.
- Warren-Wilson J, Warren-Wilson PM (1993). Mechanisms of auxin regulation of structural and physiologic polarity in plants, tissues, cells and embryos. *Aust. J. Plant Physiol.* 20:555-571.
- Webster G, Davey MR, Cocking EC (1995). *Parasponia* with rhizobia: a neglected non-legume nitrogen-fixing symbiosis. *AgBiotech News Inform* 7:119-124.
- Webster G, Gough C, Vasse J, Batchelor CA, O'Callaghan KJ, Kthari SL, Davey M R, Denarie J, Cocking EC (1997). Interactions of rhizobia with rice and wheat. *Plant Soil.*194(1-2):115-122.
- Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997). Natural endophytic association between *Rhizobium legumino-sarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant Soil* 194:99-114.
- Yanni Y G, Rizk RY, Corich V, Squatini A, Ninke K, Philip-Hollingsworth S, Orgambide G, de Bruijn, F D, Stoltzfus J, Buckley D, Schmidt T M, Mateos P F, Ladha J K, and Dazzo F B (1997). Natural endophytic associations between *Rhizobium leguminosarum* bv. *trifoli* and rice roots and assessment of its potential to promote rice growth. *Plant Soil* 194: 99-114.
- Yanni YG, Rizk RY, El-Fattah FKA, Squartini A, Corich V, Giacomini A, de Bruijn F, Rademaker J, Maya-Fores J, Ostrom P, Vega-Hernandez M, Hollingsworth RI, Martinez-Molina E, Mateos P, Velaquez E, Woperis J, Triplett E, Umali-Garcia M, Anarna JA, Rolfe BG, Ladha JK, Hill J, Mujoo R, Ng PK, Dazzo FB (2001). The beneficial plant-growth promoting association of *Rhizobium leguminosarum* bv. *Trifolii* with rice roots. *Aust. J. Plant Physiol.* 28:845-870.
- Young JM, Kuykendall LD, Martinez-Romero E, Kerr A, Sawada H (2001). A revision of *Rhizobium Frankia* 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium conn* (1942) and *Allorhizobium undicola* de Lajudie et al., 1998 as new combinations: *Rhizobium radiobacter*, R.

rhizogenes, *R. rubi*, *R. undicola* and *R. vitis*. Int J Syst Evol Microbiol 51:89–103

Zavalin AA, Vinogradova LV, Dukhanina TM, Vaulin AV, Christotin MV, Sologub DB, Gabibov M, Lekomtsev PV, Pasyukov AV (2001). Geographical regularities of effect of inoculation with associative diazotrophs on the productivity of cereals. Aspects Appl. Biol. 63:123-27.