

RICE – *RHIZOBIUM* INTERACTIONS FOR BIOLOGICAL NITROGEN FIXATION: TECHNICAL CHALLENGES

Mihirlal Roy^{1*}, Sibani Saha² and Jhuma Das³

¹Tripura State Council for Science & Technology, A.R.Complex, Agartala, Tripura, India

²Department of Fisheries, Government of Tripura, College Tilla, Agartala, Tripura, India

³IDSP, State Health and Family Welfare Society, Agartala, Tripura, India

Received-11.01.2017, Revised-24.01.2017

Abstract: Nitrogen is the most important nutrient input required for rice production. As most of the soil is deficient of N, N-fertilizers are needed. But, instead of chemical fertilisers, biological nitrogen fixation (BNF) is preferred. In that too, conventional BNF has limited capacity to render rice independent of external sources of N. Therefore, a major goal of BNF research has been to extend the nitrogen fixing capacity to rice. In this context, recent advances in understanding symbiotic *Rhizobium*-legume interactions at the molecular level, the discovery of natural endophytic interactions of rhizobacteria with rice, potentiality of rice nodulation, as well as potentiality of introduction / expression of *nif* genes in (to) rice has offered exciting opportunities to stretch rice research horizons, though there are technological challenges. These aspects have been reviewed in this article.

Keywords: Rice-*Rhizobium* interactions, Biological nitrogen fixation, Endophytic association, Nodulation in rice

REFERENCES

- Al-Mallah, M.K., Davey, M.R. and Cocking, E.C.** (1989). Formation of nodular structures on rice seedlings by rhizobia. *J. Exp. Bot.*, 40 : 473 – 478.
- Ameziane, R., Bernhard, K. and Lightfoot, D.** (2000). Expression of the bacterial *gdhA* gene encoding a NADPH-glutamate dehydrogenase in tobacco affects plant growth and development. *Plant Soil*, 221 : 47 – 57.
- Annapurna, K. and Govindasamy, V.** (2009). Rhizobia as a bioinoculant. In : *First Asian PGPR Congress for Sustainable Agriculture*, A.N.G. Ranga Agric. Univ., Hyderabad, India.
- Askary, M., Mostajeron, A., Amooghaei, R. and Mostajeron, A.** (2009). Influence of the co-inoculation of *Azospirillum brasilense* and *Rhizobium meliloti* plus 2, 4 – D on grain yield and N, P, K content of *Triticum aestivum* (cv. Baccros and Mahadevi). *American-Eurasian J. Agric. Environ. Sci.*, 5(3) : 296 – 307.
- Baset Mia, M.A., Shamsuddin, Z.H.** (2010). *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. *Afr.J.Biotechnol.*, 9(37):6001-6009.
- Bender, G.L., Preston, L., Barnard, D. and Rolfe, B.G.** (1990). Formation of nodule-like structures on the roots of the non-legumes rice and wheat. In : *Nitrogen Fixation : Achievements and Objectives* (Eds. Gresshoff, P.M., Roth, L.E., Stacey, G. and Newton, W.E.). Chapman and Hall, London, p. 825.
- Bennet, J. and Ladha, J.K.** (1992). Introduction : feasibility of nodulation and nitrogen fixation in rice. In : *Nodulation and Nitrogen Fixation in Rice : Potentials and Prospects* (Eds. Khus, G.S. and Bennet, J.), IRRI, Manila, pp. 6 – 20.
- Biswas, J.C., Ladha, J.K. and Dazzo, F.B.** (2000). Rhizobia inoculation improves nutrient uptake and growth of low land rice. *Soil. Sci. Soc. Amm.J.*, 64 : 1644 – 1650.
- Biswas, J.C., Ladha, J.K. and Dazzo, F.B.** (2000a). Rhizobial inoculation influences seedling vigour and yield of rice. *Agron. J.*, 92 : 880 – 886.
- Bottomley, P.J. and Dughri, M.H.** (1989). Population size and distribution of *Rhizobium leguminosarum* biovar *trifolii* in relation to total soil bacteria and soil depth. *Appl. Environ. Microbiol.*, 55 : 959 – 964.
- Bottomley, P.J. and Maggard, S.P.** (1990). Determination of viability within serotypes of a soil population of *Rhizobium leguminosarum* biovar *trifolii*. *Appl. Environ. Microbiol.*, 56 : 533 – 540.
- Britto, D.T. and Kronzucker, H.J.** (2001). Constancy of nitrogen turnover kinetics in the plant cell : insights into the integration of subcellular N fluxes. *Planta*, 213 : 175 – 181.
- Britto, D.T. and Kronzucker, H.J.** (2004). Bioengineering nitrogen acquisition in rice : can novel initiatives in rice genomics and physiology contribute to global food security? *BioEssays*, 26 : 683 – 692.
- Chaintreuil, C., Giraud, E., Prin, Y., Lorquin, J., Ba, A., Gillis, M, de Lajudie, P. and Dreyfus, B.** (2000). Photosynthetic *Bradyrhizobia* are natural endophytes of the Africal wild rice *Oryza breviligulata*. *Appl. Environ. Microbiol.*, 62(12) : 5437 – 5447.
- Cock, D.R., VandenBosch, K., de Bruijn, F.J. and Huguet, T.** (1997). Model legumes get the nod. *Plant Cell*, 3 : 275 – 281.
- Cocking, E.C., Al-Mallah, M.K., Benson, E., Davey, M.R.** (1990). Nodulation non-legumes by rhizobia. In : *Nitrogen Fixation : Achievements and objectives* (Eds. Gresshoff, P.M., Roth, L.E., Stacey, G. and Newton, W.E). Chapman and Hall, New York.

*Corresponding Author

- Cocking, E.C., Srivastava, J.S., Cook, J.M., Kothari, S.L. and Davey, M.R.** (1993). Studies on nodulation of maize, wheat, rice and oilseed rape : interactions of rhizobia with emerging lateral roots. In : *Biological Nitrogen Fixation – Novel Associations and Non-legume Crops* (Eds. Yanfu, N., Kennedy, I.R. and Tingwei, C.). Qingdao Ocean University Press, Qingdao, pp. 53 – 58.
- Coutinho, H.L.C., Oliveria, V.M.D and Moreira, F.M.S.** (2000). Systematics of legume nodule nitrogen fixation bacteria. In : *Applied Microbiol Systematics* (Eds. Priest, F.G. and Goodfellow, M.), Springer, New York, pp. 107 – 134.
- Dakora, F.D.** (1995). Plant flavonoids : biological molecules for useful exploitation. *Aust. J. Plant Physiol.*, 22 : 7 – 99.
- Dey, B. and Srivastava, R.C.** (2001). Biodiversity of *Rhizobium* species in respect of their growth and nitrogen-fixing efficiency in free-living culture. *Vegetos*, 14 : 45 – 49.
- de Bruijn, F. J., Jing, Y. and Dazzo, F.B.** (1995). Potential and pitfalls of trying to extend symbiotic interactions of nitrogen-fixing organisms to presently non-nodulated plants, such as rice. *Plant Soil*, 172 : 207 – 219.
- Dixon, R., Cheng, Q., Shen, G.F., Day, A. and Dowson-Day, M.** (1997). *Nif* gene transfer and expression in chloroplasts : prospects and problems. *Plant Soil*, 194 : 193 – 203.
- Dixon, R., Cheng, Q., Day, A.** (2000). Prospects for constructing nitrogen-fixing cereals. In : *The Quest for Nitrogen Fixation in Rice* (Eds. Ladha, J.K. and Reddy, P.M.), Proceedings of the Third Working Group Meeting of Assessing Opportunities for Nitrogen Fixation in Rice, IRRI, Manila, pp. 327 – 336.
- Engelhard, M., Hurek, T. and Reinhold-Hurek, B.** (2000). Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp. in wild rice species and land races of *Oryza sativa* in comparison with modern races. *Environ. Microbiol.*, 2 : 131 – 141.
- Fischer, K.S.** (2000). Frontier project on nitrogen fixation in rice : looking ahead. In : *The Quest for Nitrogen Fixation in Rice* (Eds. Ladha, J.K. and Reddy, P.M.), Proceedings of the Third Working Group Meeting of Assessing Opportunities for Nitrogen Fixation in Rice, IRRI, Manila, pp. 25 – 32.
- Fred, E.B., Baldwin, I.L., McCoy, E.** (1932). Root nodule bacteria and leguminous plants. University of Wisconsin Press, Madison, p. 343.
- G-Laboratory, China** (2002). Research on biological nitrogen fixation with non-legumes. In : *9th International Symposium on Nitrogen Fixation with Non-legumes*, Leuven, Belgium.
- Gallardo, F., Fu, J.M., Canton, F.R., Garcia-Gutierrez, A. and Canvas, F.M.** (1999). Expression of a conifer glutamine synthetase gene in transgenic poplar. *Planta*, 210 : 19 – 26.
- Geurts, R. and Bisseling, T.** (2002). *Rhizobium* Nod factor perception and signalling. *Plant Cell*, 14 : S239 – S249.
- Gianinazzi-Pearson, V.** (1996). Plant cell responses to arbuscular mycorrhizal fungi : getting to the roots of the symbiosis. *Plant Cell*, 8 : 1871 – 1883.
- Graham, P.H.** (1988). Biological nitrogen fixation : symbiotic. In : *Principles and Application of Soil Microbiology* (Eds. Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G. and Zuberer, D.A.), Prentice-Hall, Inc., Englewood Cliffs, N.J., pp. 322 – 345.
- Gray, E.J. and Smith, D.L.** (2005). Intracellular and extracellular PGPR : commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol. Biochem.*, 37 : 395 – 412.
- Habash, D.Z., Massiah, A.J., Rong, H.L., Wallsgrave, R.M. and Leigh, R.A.** (2001). The role of cytosolic glutamine synthetase in wheat. *Ann. Appl. Biol.*, 138 : 83 – 89.
- Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I.** (2010). Soil beneficial bacteria and their role in plant growth promotion : a review. *Ann. Microbiol.*, 60(4) : 579 – 598.
- James, E.K.** (2000). Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res.*, 65 : 197 – 209.
- Jing, Y., Li, G., Jin, G., Shan, X., Zhang, B., Guan, C. and Li, J.** (1990). Rice root nodules with acetylene reduction activity. In : *Nitrogen Fixation : Achievements and Objectives* (Eds. Gresshoff, P.M., Roth, L.E., Stacey, G. and Newton, W.E.). Chapman and Hall, London, p. 829.
- Jing, Y., Li, G. and Shan, X.** (1992). Development of nodule-like structure on rice roots. In : *Nodulation and Nitrogen Fixation in Rice : Potentials and Prospects* (Eds. Khush, G.S. and Bennett, J.). IRRI, Manila, pp. 123 – 126.
- Kennedy, I.R., Pereg-Gerk, L.L., Wood, C., Deaker, R., Gilchrist, K. and Katupitiya, S.** (1997). Biological nitrogen fixation in non-leguminous field crops : facilitating the evolution of an effective association between *Azospirillum* and Wheat. *Plant Soil*, 194 : 65 – 79.
- Kennedy, I.R., Chowdhury, A.T.M.A. and Kecskes, M.L.** (2004). Non-symbiotic bacterial diazotrophs in crop-farming system : can there potential for plant growth promotion be better exploited ? *Soil Biol. Biochem.*, 36 : 1229 – 1244.
- Keyeo, F., Noor Ai'shah, O. and Amir, H.G.** (2011). The effects on nitrogen fixation activity and phytohormone production of diazotroph in promoting growth of rice seedlings. *Biotechnol.*, 10(3) : 267 – 273.
- Keyser, H.H. and Li, F.** (1992). Potential for increasing biological nitrogen fixation in soybean. *Plant Soil*, 141 : 119 – 135.
- Khalid, A., Arshad, M. and Zahir, Z.A.** (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.*, 96 : 473 – 480.

- Khan, A.G. and Belik, M.** (1995). Occurrence and ecological significance of mycorrhizal symbiosis in aquatic plants. In : *Mycorrhiza* (Eds. Verma, A. and Hook, B.), Springer-Verlag, Berlin, pp. 627 – 666.
- Khan, A.G.** (2005). Role of soil microbes in the rhizosphere of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace Elem. Med. Biol.*, 18 : 355 – 364.
- Ladha, J.K., Garcia, M., Miyay, S., Padre, A.T. and Watanabe, I.** (1989). Survival of *Azorhizobium caulinodans* in the soil and rhizosphere of wetland rice under *Sesbania rostrata* rice rotation. *Appl. Environ. Microbiol.*, 55 : 454 – 460.
- Ladha, J.K. and Peoples, M.B.** (1995). Management of biological nitrogen fixation for the development of more productive and sustainable agricultural systems. *Plant Soil*, 174 : 1 – 286.
- Ladha, J.K., So, R., Hernandez, R., Dazzo, F.B., Reddy, P.M., Angeles, O.R., Ramos, M.C., de Bruijn F.J. and Stoltzfus, J.** (1996). Rhizobial invasion and induction of phenotypic changes in rice roots are independent of Nod factors. In : *Proceedings 8th International Congress on Molecular Plant-Microbe Interactions* (Abstr.#L – 11), Knoxville, TN.
- Ladha, J.K., de Bruijn, F.J. and Malik, K.A.** (1997). Introduction : assessing opportunities for nitrogen fixation in rice – a frontier project. *Plant Soil*, 194 : 1- 10.
- Lhuissier, F.G.P., de Ruijter, N.C.A., Sieberer, B.J., Esseling, J.J. and Emons, A.M.C.** (2001). Time course of cell biological events evoked in legume root hairs by *Rhizobium* nod factors : state of the art. *Ann. Bot.*, 87 : 289 – 302.
- Li, G., Jing, Y., Shan, X., Wang, H. and Guan, C.** (1991). Identification of rice nodules that contain *Rhizobium* bacterium. *Chin. J. Bot.*, 3 : 8 – 17.
- Lucy, M., Reed, E. and Glick, B.R.** (2004). Applications of free-living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek*, 86 : 1- 25.
- Matiru, V.N. and Dakora, F.D.** (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.
- Merrick, M. and Dixon, R.** (1984). Why don't plants fix nitrogen? *Trends Biotechnol.*, 2 : 162 – 166.
- Okon, Y.P.G.** (1985). *Azospirillum* as a potential inoculant for agriculture. *TIBTech.*, 3 : 223 – 228.
- Parakaran, J.** (1997). BNF rice : the choice of new generations. In : *Proceedings of the Plant Genetic Seminar on Nitrogen Fixation*, Univ. of Wisconsin at Madison, Wisconsin, USA.
- Patel, S. and Sinha, S.** (2011). Rhizobia species : a boon for 'Plant Genetic Engineering'. *I.J. Microbiol.*, 51(4) : 521 – 527.
- Plazinski, J., Innes, R.W. and Rolfe, B.G.** (1985). Expression of *Rhizobium trifolii* early nodulation genes on maize and rice plants. *J. Bacteriol.*, 163 : 812 – 815.
- Postgate, J.** (1998). Nitrogen Fixation. Cambridge Univ. Press, Cambridge, 3rd edn, pp. 112.
- Raj, N.S., Chalubaraju, G., Amruthesh, K.N., Shetty, H.S., Reddy, M.S. and Kloepper, J.W.** (2003). Induction of growth promotion and resistance against downy mildew on pearl millet (*Pennisetum glaucum*) by rhizobacteria. *Plant Dis.*, 87 : 380 – 384.
- Reddy, P.M., Kouchi, H., Hata, S. and Ladha, J.K.** (1996). Homologs of *GmENOD93* from rice. In : *8th International Congress on Molecular Plant-Microbe Interactions*, Knoxville, TN.
- Reddy, P.M., Kouchi, H., Hata, S. and Ladha, J.K.** (1996a). Identification, cloning and expression of rice homologs of *GmN93*. In : *7th International Symposium on BNF with Non-Legumes*, Faisalabad.
- Reddy, P.M., Ladja, J.K., So, R.B., Hernandez, R.J., Ramos, M.C. and Angeles, O.R.** (1997). Rhizobial communication with rice roots : induction of phenotypic changes, mode of invasion and extent of colonization. *Plant and Soil*, 194 : 81 – 98.
- Reddy, P.M., Ladha, J.K., Kouchi, H., Stacey, G., Hernandez-Oane, R.J., Ramos, M.C., So, R.B., Angeles, O.R., Sreevidya, V.S., Day, R.B., Cohn, J. and Koh, S.** (2000). Realizing the genetic predisposition of rice for symbiotic nitrogen fixation. In : *The Quest for Nitrogen Fixation in Rice* (Eds. Ladha, J.K. and Reddy, P.M.), IRRI, Philippines, pp. 241 – 261.
- Ribbe, M., Gadkari, D. and Meyer, O.** (1997). N₂ fixation by *Streptomyces thermoautotrophicus* involves a molybdenum-dinitrogenase and a manganese-superoxide oxidoreductase that couple N₂ reduction to the oxidation of superoxide produced from O₂ by a molybdenum-CO dehydrogenase. *J. Biol. Chem.*, 272 : 26627 – 26633.
- Rolfe, B.G., Bender, G.L.** (1990). Evolving a *Rhizobium* for non-legume nodulation. In : *Nitrogen Fixation : Achievements and Objectives* (Eds. Gresshiff, P.M., Roth, L.E., Stacey, G. and Newton, W.E.). Chapman and Hall, London, pp. 779 – 786.
- Roy, M.L. and Srivastava, R.C.** (2010). Single and co-inoculation effects of different biofertilizers on growth, *in vivo* nitrate reductase activity and soluble protein in *Oryza sativa* L. *J. Appl. Biosci.*, 36(1) : 101 – 104.
- Roy, M.L.** (2013). Influence of inoculation of selected nitrogen fixing biofertilizers on certain physiological and biochemical parameters, growth and yield performance of rice cultivars. Ph.D Thesis, Tripura University, India.
- Sahgal, M and Johri, B.N.** (2003). The changing face of rhizobial systematic. *Curr. Sci.*, 84(1) : 43 – 48.
- Saikia, S.P. and Jain, V.** (2007). Biological nitrogen fixation with non-legumes : a achievable target or a dogma? *Curr. Sci.*, 92(3) : 317 – 322.
- Schell, J., Schmidt, J., John, M. and Rohrig, H.** (1992). Potential and limitations of developing new plant-microbe interactions. In : *Nodulation and*

- Nitrogen Fixation in Rice* (Eds. Khush, G.S. and Bennet, J.), IRRI, Phillipines, pp. 103 – 105.
- Secilia, J. and Bagyaraj, D.J.** (1992). Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice (*Oryza sativa* L.). *Biol. Fert. Soils*, 13 : 108 – 111.
- Shantharam, S. and Mattoo, A.K.** (1997). Enhancing biological nitrogen fixation : an appraisal of current and alternative technologies for N input into plants. *Plant Soil*, 194 : 205 – 216.
- Sharma, A.K.** (2003). Microbial inoculants for nitrogen fixation. In : *Biofertilizers for Sustainable Agriculture*, Agrobios, India, pp. 85 – 155.
- Sofi, P. and Wani, S.** (2007). Prospects of nitrogen fixation in rice. *Asian J. Plant Sci.*, 6 : 203 – 213.
- Spaink, H.P. and Lugtenberg, B.J.J.** (1992). Application of present knowledge on rhizobial host specificity to obtain efficient nodulation and nitrogen fixation of rice. In : *Nodulation and Nitrogen Fixation in Rice* (Eds. Khush, G.S. and Bennet, J.), IRRI, Phillipines, pp. 107 – 110.
- Stoltzfus, J.R., So, R., Malarvithi, P.P., Ladha, J.K. and de Bruijn, F.J.** (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant and Soil*, 194 : 25 – 36.
- Stougaard, J.** (2001). Genetics and genomics of root symbiosis. *Curr. Opin. Plant Biol.*, 4 : 328 – 335.
- Subba Rao, N.S.** (1982). *Soil Microorganisms and Plant Growth*. Oxford and IBH Pub. Co., New Delhi, p. 289.
- Terouchi, N and Syono, K.** (1990). *Rhizobium* attachment and curling in asparagus, rice and oat plants. *Plant Cell Physiol.*, 31 : 119 – 127.
- Trevaskis, B., Colebatch, G., Desbrosses, G., Wadrey, M. and Wienkoop, S.** (2002). Differentiation of plant cells during symbiotic nitrogen fixation. *Comp. Func. Gen.*, 3 : 151 – 157.
- Van Berkum, P. and Bohlool, B.B.** (1980). Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. *Microbiol. Rev.*, 44 : 491 – 517.
- Van Loon, L.C.** (2007). Plant responses to plant growth-promoting bacteria. *Eur. J. Plant Pathol.*, 119 : 243 – 254.
- Webster, G., Gough, C., Vase, J., Bachelor, C.A., O'Callaghan, K.J., Kothari, S.L., Davey, M.R., Denarik, J. and Cocking, E.C.** (1997). Interactions of rhizobia with rice and wheat. *Plant and Soil*, 194 : 115 – 122.
- Weir, B.S.** (2012). The current taxonomy of rhizobia. NZ Rhizobia website. <http://www.rhizobia.co.nz> / taxonomy / rhizobia (updated as on April 10, 2012).
- Whitfed, P.R. and Bottomley, W.** (1983). Organization and structure of chloroplast genes. *Ann. Rev. Plant Physiol.*, 34 : 297 – 310.
- Yanni, Y. G. and Dazzo, F. B.** (2010). Enhancement of rice production using endophytic strains of *Rhizobium leguminosarum* bv. *trifolii* in extensive field inoculation trials within the Egypt Nile delta. *Plant Soil*, 336 (1 – 2) : 129 – 142.
- Yanni, Y.G., Rizk, E.Y., Conch, V., Squartini, A., Ninke, K., Philip – Hollingsworth, S., Orgambide, C.G., de Bruijn, F.J., Stoltzfus, J., Buckley, D., Schmidt, T.M., Matcos, P.F., Ladha, J.K. and Dazzo, F. B.** (1997). Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant Soil*, 194 : 99 – 114.
- Yanni, Y.G. and Rizk, R.Y., Abd EI – Fattah, F.K., Squartini, A., Corich, V., Giacomni, A., de Bruijn, F., Rademaker, J., Maya – Flores, J., Ostrom, P., Vega-Hernandez, M., Hollingsworth, R.I., Martinez – Molina, E., Triplett, E., Umali-Garcia, M., Anarna, J.A., Rolfe, B.G., Ladha, J.K., Hill, J., Mujoo, R., Ng, P.K. and Dazzo, F.B.** (2001). The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. *trifolii* with rice roots. *Aust. J. Plant Physiol.*, 28 : 845 – 870.