ROLE OF RHIZOBIAL ENDOPHYTES AS NITROGEN FIXER IN PROMOTING PLANT GROWTH AND PRODUCTIVITY OF INDIAN CULTIVATED UPLAND RICE (*ORYZA SATIVA* L.) PLANTS

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Rhizobia are bonafide member of symbiotic N_2-fixing microorganisms. They can exclusively induce root or stem nodules on leguminous hosts. Recently it has been explored that rhizobia can make an intimate association with non-legumes such as rice, wheat, maize, etc as endophyte, without forming any nodule like structure or causing any disease symptoms (Yanni *et a*l 1997; Chaintreuil *et al*., 2000; Gutierrez-Zamora and Martinez-Romero 2001; Tan *et al* 2001). The infection process of rhizobia to rice is *nod* gene independent, non-specific and does not involve the formation of infection threads which is a characteristic feature of legume-rhizobia symbiosis. Although rhizobial colonization extend from rhizosphere to inner into the epidermis, endodermis and cortex, but the main site of colonization is intercellular spaces of rice root (Reddy *et al* 1997). Rhizobial interaction with rice may be manifested a full spectrum of growth responses. Rhizobia may promote, inhibit or have no effect on rice plant growth (Yanni *et al*, 2001, Prayitno *et al*., 1999; Perrine et al., 2001 Biswas *et al* 2000). In this study, we have reported the isolation of rhizobial endophytes from rice roots of cultivated upland Indian rice plant and their ability to fix nitrogen in association with the rice as host.

Materials and Methods

Rice root samples were collected at heading stage. Roots were thoroughly washed and surface sterilized as described by Yanni *et al*., (1997). Samples were macerated aseptically and one ml of suspension was inoculated to a number of leguminous plants like, *Trifolium, Phaseolus, Cicer, Vigna, Sesbania, Pisum,* etc. Data on nodule induction was recorded after 45 days of inoculation. In order to study endophytic colonization of rice root the best performing rhizobial isolate (BHUE-3) was conjugated with *E. coli* strain S17-1lambda pir (m*Tn*5S*gus*A21) harboring *gus*A reporter gene. Gus tagged rhizobia was inoculated to axenically growing rice plants. Gus assay was carried out after 30 days of inoculation according to Verma *et al*, (2001). Plant growth contributing

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factors like nitrogen fixation, phosphate solubilization, phytohormone production and secretion of cell wall degrading enzymes were determined for rhizobial isolates. Nitrogenase activity was measured according to Hardy *et al.* (1973). Phosphate solubilization, cellulase and pectinase activities were detected in YEM plates supplemented with $CaHPO_4$, corboxymethyl cellulose (CMC) and pectin, respectively (Verma *et al*, 2001; Yanni *et al*., 2001). Green house experiment was performed in pots containing oven dried field soil. Plants were inoculated with 1 ml (~10^8 CFU) rhizobial culture and observation was recorded related to plant growth at maturity. Number of replications was six for each treatment and statistical analyses were done in Microsoft EXCEL computer software package.

Results and Discussion

Using the legume trap technique, we have isolated nine endophytic rhizobial strains and designated them as BHUE-1 to BHUE-9. These isolates followed the Koch postulates and expressed the ability to nodulate commonbean (*Phaseolus vulgaris* L.) plants under axenic conditions. Out of the nine isolates, four (BHUE-2, 3, 4 and BHUE-5) were selected for plant growth response studies under laboratory and green house conditions. All four isolates gave a positive response in enhancing the plant growth measured in terms of plant height and shoot dry weight. BHUE-3 and BHUE-5 showed the best response (Table 1). In addition to other plant growth promotion mechanisms such as phosphate solubilization and phytohormone production, these isolates expressed nitrogenase activity both *ex planta* and in association with rice plants. Such activity was not detected when plants inoculated with USDA 2695 or ANU843 were used for assay.

Table 1. Effect of rhizobial inoculation on growth and productivity of rice (cv Sarjoo-52) in green house conditions.

Strains	Characters			
	Plant height (cm)	Shoot dry weight (g $hill^{-1}$)	Grain yield (g $hill^{-1}$)	Nitrogenase activity §
Control	89.8	20.76	12.98	Nil
BHUE2	94.6^{ns}	24.72**	14.4*	0.81 ± 0.34*
BHUE3	96.0*	25.65**	15.53**	0.96 ± 0.42
BHUE4	93.1^{ns}	22.60*	14.25*	0.84 ± 0.40
BHUE5	95.3*	26.80**	15.60**	0.92 ± 0.38
USDA2695	92.2^{ns}	21.75^{ns}	13.32^{ns}	Nil
ANU843	91.6^{ns}	21.68^{ns}	13.00^{ns}	Nil

§ (micro mol C_2H_4 g^{-1} dry weight h^{-1})

Mean values followed by *or ** are significantly higher than control at the 95% and 99% levels, respectively and 'ns' indicates non-significant.

Values followed by ± indicates standard error mean

Although ANU843 is known to promote rice plant growth, its inability to express nitrogenase activity with rice cultivar Sarjoo 52 indicate that the conditions required for nitrogenase activity is not available whereas in the similar condition, the expression of nitrogen fixation using endophytic rhizobial isolates as source of inoculation indicate that these isolates create a better atmosphere for expression of *nif* genes.

The expression *nif* gene in "rice born" rhizobial inside the rice root and in free-living conditions also indicates that these isolates might be having some additional gene(s) that provide a protection for their nitrogenase enzymes from oxygen damage. Mutational and transcriptional analysis of nitrogen fixing machinery of these isolates will provide more authentic evidences for endophytic colonization, nitrogen fixation and plant growth promotion.

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