Influence of rhizobium on the growth and yield of rice

Business, Industries

Title

Influence of Rhizobium on the growth and yield of Rice

Abstract

Rice (Oryza sativa L.) is one of the world's most important crops. The present investigation was designed to assess the range of growth-promoting activities of various diazotrophic bacteria on rice seedling vigor, its carryover effect on straw and grain yield, and the persistence of an inoculant strain on rice roots under greenhouse conditions.

Growth responses to inoculation exhibited bacterial strain–rice variety specificity that were either stimulatory or inhibitory. Growth responses included changes in rates of seedling emergence, radical elongation, height and dry matter, plumule length, cumulative leaf and root areas, and grain and straw yields. Most notable were the inoculation responses toRhizobium leguminosarum bv. trifolii E11 and Rhizobium sp.

IRBG74, which stimulated early rice growth resulting in a carryover effect of significantly (P = 0. 05) increased grain and straw yields at maturity, even though their culturable populations on roots diminished to below detectable values at 60 d after planting. The test strains were positive for indole-3-acetic acid production in vitro, but only some reduced acetylene to ethylene in association with rice under laboratory growth conditions.

These studies indicate that certain strains of nonphotosynthetic diazotrophs, including rhizobia, can promote growth and vigor of rice seedlings, and this benefit of early seedling development can carryover to significantly increased grain yield at maturity. Rhizobia, the root-nodule endosymbionts of

leguminous plants, also form natural endophytic associations with roots of important cereal plants. Despite its widespread occurrence, much remains unknown about colonization of cereals by rhizobia.

We examined the infection, dissemination, and colonization of healthy rice plant tissues by four species of gfp-tagged rhizobia and their influence on the growth physiology of rice. The results indicated a dynamic infection process beginning with surface colonization of the rhizoplane (especially at lateral root emergence), followed by endophytic colonization within roots, and then ascending endophytic migration into the stem base, leaf sheath, and leaves where they developed high populations. In situ CMEIAS image analysis indicated local endophytic population densities reaching as high as 9 ? 010 rhizobia per cm3 of infected host tissues, whereas plating experiments indicated rapid, transient or persistent growth depending on the rhizobial strain and rice tissue examined. Rice plants inoculated with certain test strains of gfp-tagged rhizobia produced significantly higher root and shoot biomass; increased their photosynthetic rate, stomatal conductance, transpiration velocity, water utilization efficiency, and flag leaf area (considered to possess the highest photosynthetic activity); and accumulated higher levels of indoleacetic acid and gibberellin growth-regulating phytohormones.

Considered collectively, the results indicate that this endophytic plant-bacterium association is far more inclusive, invasive, and dynamic than previously thought, including dissemination in both below-ground and above-ground tissues and enhancement of growth physiology by several rhizobial

species, therefore heightening its interest and potential value as a biofertilizer strategy for sustainable agriculture to produce the world's most important cereal crops.

Introdution

Greater production of cereals brings forth higher production cost and pollutes the soilenvironmentdue 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 biofertilizertechnology, 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, barleymillets 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 nonlegumes 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 prThe United NationsFoodand 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.

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 somehow 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 Kuppuswamy, 2001).

Inoculation with bacterial biofertilizer may reduce the application 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. 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. Host plants Rhizobia Colonization Growing condition 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). Materials and Methods____

Rhizobium strains and plasmids used in this study are listed in Table1. The vector pHC60 encodes for tetracycline resistance and contains the gfp gene that is constitutively expressed at fairly constant levels from a constitutive lacZ promoter without the required expression of lacZ.

This vector was similar to the one used earlier by Gage et al, except that it contains a stability region so that expression of its gfp is more stably maintained within bacterial cells in the absence of selective pressure . The half-life of the green fluorescent protein (GFP) in some organisms is approximately 1 day. For construction of the gfp-tagged strains, the pHC60 vector was transferred to the wild-type rhizobia strains using the triparental mating method. Japonica rice (Oryza sativa L. varieties Zhonghua 8 and Nipponbare were obtained from the Institute of CropScience, Chinese Academy of Agriculture, China, and National Institute of Agrobiological Sciences of Japan. Medicago sativa L. , Sesbania rostrata, Astragalus

sinicus L. , and Pisum sativum L. were used as the legume hosts. TABLE 1 Plasmids and bacterial strains used in this study | Plasmid or Rhizobium strain | Characteristics (amt of | Source | | | antibiotic used [? /ml])a | | | pHC60 | Broad-host-range plasmid | City University of New York (6) | | | with gfp; Tcr (10) | | | Sinorhizobium meliloti 1021 | Smr (50) | Institute of Plant Physiology, Shanghai, People's Republic | | | | of China | | Azorhizobium caulinodans ORS 571 | Ampr (100), Cbr (100) | Academy of Agriculture, Beijing, People's Republic of China | | Sinorhizobium meliloti USDA 1002 | Kmr (50) | Chinese University of Agriculture, Beijing, People's | | | Republic of China | | Rhizobium leguminosarum USDA 2370 | Smr (50) | Chinese University of Agriculture, Beijing, People's | | | | Republic of China | | Mesorhizobium huakui 93 | Smr (10) | Nanjing University of Agriculture, Nanjing, People's | | | | Republic of China | | Sinorhizobium meliloti 1021 (gfp) | Smr (50), Tcr (10) | City University of New York, New York, N. Y. | | Azorhizobium caulinodans ORS 571 (gfp) | Ampr (100), Tcr (10) | This study | | Sinorhizobium meliloti USDA 1002 (gfp) | Kmr (50), Tcr (10) | This study | | Rhizobium leguminosarum USDA 2370 (gfp) | Smr (50), Tcr (10) | This study | | Mesorhizobium huakui 93 (gfp) | Smr (10), Tcr (10) | This study | Tcr, tetracycline resistance; Smr, streptomycin resistance; Cbr, carbenicillin resistance; Kmr, kanamycin resistance; Ampr, ampicillin resistance; Tcr, tetracycline resistance Growth conditions: For gnotobioticcultureof rice with rhizobia, seeds were de-hulled, treated with 70% ethanol for 10 min, washed three times with sterile water, surface sterilized with 0. 1% HgCl2 for 10 min, and then washed three times again with sterile water. Surface-sterilized seeds were germinated on Luria-Bertani

(LB) plates in the dark for 2 days at 28°C to verify that they were axenic. Four to five small axenic seedlings were transferred aseptically into sterilized glass tubes (40 cm in height, 5 cm in diameter) containing 250 cm3 of sterile vermiculite and 125 ml of half-strength Hoagland's no. 2 plant growth medium.

Bacteria were cultured for 2 days at 28°C on TY agar containing the appropriate antibiotic(s), and then suspended in phosphate-buffered saline (PBS; pH 7. 4) to 108 cells/ml. A total of 5 ml of bacterial inoculum was carefully introduced into the seedling rhizosphere using a pipette tip inserted 1 cm below the vermiculite surface in each tube in order to avoid contamination of the above-ground rice tissues. Each inoculated tube was covered with transparent tissue culture paper (12 cm by 12 cm) and incubated in a growth chamber programmed with a 14-h photoperiod and 28/25°C day/night cycle. Three inoculated seedlings were replicated for each strain.

GFP-tagged rhizobia recovered from rice were tested for nodulation ability on their respective legume host in a gnotobiotic tube culture. Conditions were the same as described above for rice except that the gnotobiotic tube cultures contained 100 cm3 of vermiculite and 60 ml of Fahraeus nitrogen-free nutrient medium, and the rhizobial inoculum density was 106 cells/ml. At 30 days postinoculation (dpi) in the growth chamber, the roots were checked for nodules that exhibited green fluorescence. For potted soil culture of rice with rhizobia, seeds (without surface sterilization) were

imbibed in tap water for 2 days, and then transferred to a small pot containing watered soil.

After 20 days of growth under outdoor ambient conditions, the seedlings were transplanted to large pots, each containing 13 liters of watered, nonsterilized porous media (soil-vermiculite mix [1: 1]). After 7 and 45days of growth, the seedlings were inoculated with 100 ml of the same rhizobial cell suspension (optical density at 600 nm = 0. 8) into each pot (three replicates per strain), being careful not to contaminate above-ground plant tissues. Potted plants were grown in the open outdoors for 160 dpi before harvesting. Microscopy and image analysis: The GFP accumulated within the gfp-tagged bacteria produces a sufficiently bright fluorescent signal allowing for their single-cell detection and quantification en masse by computer-assisted fluorescence microscopy.

Since all the rhizobial cells carry the plasmid and express the gfp gene at approximately the same level, their local population sizes can be analyzed by measuring the integrated density of emitted fluorescent light from within a defined area or volume. Tissues of rice roots, leaf sheaths at the leaf base, and leaves (Fig. 1) were excised from plants removed from tubes at regular intervals and rolled over TY plates to check whether bacteria could be cultured from their external surfaces. The knife was washed between cuttings with sterile water and 70% alcohol and wiped with sterile absorbent paper to avoid cross-contamination of tissues during excision.

Free-hand sections of the excised tissues were rinsed clean with sterile water, mounted on slides and examined using a Bio-Rad MRC 1024 laser

confocal microscope with 488- and 568-nm band-pass filters to capture the green fluorescence from gfp-tagged bacteria and the red autofluorescence from host tissue, respectively. The images were acquired as confocal Z-section series using a Nikon E800 scanner and digital camera and then merged into loss-less montage images using Confocal Assistant Software. Only regions of infected tissues and plant cells immediately surrounding them located at least 20 ? m beneath the cut surfaces were included in these extended focus images prepared from Z-series of optisections used to measure the local cell density of endophytic bacteria.

This sampling design excluded any fluorescent bacteria lying directly on the cut surface that may have been redistributed while sectioning the tissues and extraneous tissue inaccessible to the bacteria in order to achieve as high of a signal-to-noise ratio of input data as possible for calculating the local population density. Various rice tissues used in these studies. Fig. 1. Images were spatially calibrated, color segmented, and analyzed using CMEIAS Image Analysis software to measure the local population density and spatial distribution of fluorescent gfp-tagged bacteria in situ within the plant tissues. The voxel dimensions were computed from the two-dimensional projected area of fluorescent bacteria within the Z-series of image optisections containing infected tissue, the cumulative Z thickness of each image stack, and 0. 5 ? 3 as the average biovolume of each vegetative rhizobial cell (computed from an image analysis of 10 individual, well-isolated bacteria in planta). Geostatistical analysis was performed on confocal images to examine the connectivity between local population density and spatial distribution of the endophytic bacteria. For these analyses, each digital

image was processed by CMEIAS Color Segmentation software to create a new image containing all of the foreground pixels that represent the fluorescent green microbes of interest on a noise-free background and then digitally cut into 144 square quadrats created by a 12-by-12 grid overlay using CMEIAS Quadrat Maker software.

Each image quadrat was then analyzed using CMEIAS Image Analysis software to extract its object count as the Z variate, and the Cartesian x and y coordinates of its representative sample posting within the original image, weighted by the x and y coordinate positions of the objects' local density within it . These plot-based, georeferenced data of local bacterial density were tested for spatial autocorrelation, and the derived geostatistical model that best fits the computed spatial variance in local density was then used to compute the spatial scale in which bacteria influence each other's distribution in situ and the corresponding two-dimensional kriging interpolation map of this regional variable over the same spatial domain. Viable plating experiments: The rice roots from gnotobiotic seedlings were carefully removed from each tube, excised, washed with sterile water, blotted dry, divided into two parts, and weighed.

One portion (1: 20 wt/vol) was surface sterilized by vortexing for 1 min in a solution of 1% bleach, 0. 1% sodium dodecyl sulfate, and 0. 2% Tween 20 in PBS. These samples were then rinsed four times with sterile water, placed on agar plates of LB medium for 1 h, and then removed. These plates developed no colonies when incubated for 2 days at 28°C, verifying that the excised roots were surface sterilized. To enumerate the endophytic rhizobia, excised

surface-sterilized roots were macerated with a sterile mortar and pestle, diluted in PBS solution containing 20% glycerol, and spread on TY plates supplemented with tetracycline (10 ? g/ml) and the other appropriate antibiotic(s) for each test strain.

The other excised roots were macerated without surface sterilization and plated as described above to enumerate the combined, viable populations of the rhizobial test strain on the root surface and internal tissue. To verify that the test strains had not contaminated aerial plant surfaces in these gnotobiotic cultures, fragments of leaf sheaths at the leaf base and 2-cm fragments of leaves were transferred directly without prior surface sterilization onto LB plates for 1 h, and then the plates were incubated at 28°C. After removal from the LB plates, the excised plant tissues were macerated, diluted in PBS, and plated out as described above to enumerate the endophytic population of viable bacterial cells.

To enumerate the viable endophytic rhizobia within tissues of rice grown for 125 days in open potted soil, 15 cm of the leaf sheaths and 15 cm of the leaf fragments were excised 5 and 20 cm, respectively, above the taproots (Fig1) from three replicate plants per treatment. These excised tissues were surface sterilized separately in 15% NaOCl for 10 min, washed four times with sterile water, and rolled over the surface of LB solid medium as a test to check whether their surfaces were free of contamination. The tissues were then homogenized in sterile PBS-glycerol and centrifuged briefly at slow speed (785 g) to remove the bulk of macerated plant tissue without affecting the colony counts of suspended bacteria.

The supernatant suspensions were diluted in sterile PBS and plated on TY plates containing tetracycline plus the appropriate antibiotic for each rhizobial test strain (Table1). After 2 days of incubation at 28°C, the colonies were counted from each of the three replicate tissue samples, and 10 colonies from each sample set were checked for green autofluorescence using fluorescence microscopy and nodulation ability on the homologous legume host in gnotobiotic culture as described above. Analysis of rice growth responses after inoculation with rhizobia: Axenic seedlings derived from surface-sterilized seeds were transferred to pots containing 13 liters of nonsterilized and watered porous media (soil-vermiculite [1: 1]) and grown outdoors.

Each pot was planted with 18 seedlings, replicated in three pots, and inoculated with a single strain. The inoculum for each pot consisted of rhizobial cells cultured in TY liquid medium, suspended in 100 ml of PBS buffer to a density of optical density at 600 nm of 0. 8, and poured into each planted pot. After a short period of growth, seedlings were tillered to 2 to 3 plants that ultimately produced around 45 to 54 plants per pot at maturity. After 130 days of growth, five top flag leaves of the rice plants selected from each replicate of three pots were analyzed nondestructively for net photosynthetic rates using a portable photosynthesis system LI-COR6400 (LICOR Biosciences) according to the manufacturer's instructions.

Simultaneously, the stomatal conductance, transpiration velocity, and CO_2 concentration within the flag leaves were recorded using the same instrument. Plants were then harvested after 160 days of rice growth. The

mean height was measured from the above-ground stem base to the top leaf without the spike from a total of 135 to 162 rice plants (three pots). Mean fresh weight, biovolume, dry weight, nitrogen content, and seed yield of plants per pot were calculated as the averages of the three replicate pots. Root biovolume was measured using water displacement. Plant dry weight was measured after drying in an oven for 2 days at 80°C. Total nitrogen content was measured by the Kjeldahl method, and total grain yield was calculated as the mean seed weight times the total seed yield per pot.

Review of literature

Rice-adapted isolates of rhizobia have previously been shown to produce the growth-regulating phytohormones IAA and GA in pure culture and increase IAA levels accumulated externally in root exudate of gnotobiotically cultured rice plants. Our finding that endophytic rhizobia disseminate from below-ground into above-ground rice tissues prompted us to examine whether the levels of growth-regulating phytohormones within these tissues are influenced by the internal, ascending rhizobia. High-pressure liquid chromatography analyses indicated elevated levels of IAA and GA3 extracted from the leaf sheaths and leaves of rice plants whose rhizospheres were inoculated with S. meliloti 1021 and A. caulinodans ORS571, respectively.

We predict that this rhizobium-induced elevation of the levels of these growth-stimulating phytohormones within the above-ground rice tissues contributes to the underlying mechanism(s) allowing certain strains of these bacteria to enhance vegetative and reproductive growth of cereals in general. 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 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 gro inoculation of Rhizobium in difinoculated plants are shifted to the field. Some of the factors that may affect the performance of inoculums are soil type, organic matter and other soil physical factors. High concentration of Nfertilizers especially NH4NO3 change the morphology and activity of N2 fixing bacteria consequently causing harmful effects. Yield and nodulation were found to have significant positive correlation for both crop reasons. For the bean-sorghum intercropped conditions, the use of mixed granular rhizobial inoculants 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 iofertilizers 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 byferent cereal grains8 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 droughtstress. 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). Results and discussion____ Microscopy of rice tissues colonized with gfp-tagged Sinorhizobium meliloti 1021: Roots examined at six dpi contained fluorescent green cells of gfp-tagged S. eliloti 1021 preferentially colonized around lateral root junctions, where they gained entry into the root interior between displaced epidermal cells, a finding consistent with previous studies indicating this as the major site of colonization on epidermal root surfaces and primary host infection in cereals by rhizobia. By 10 to 14 dpi, the fluorescent bacteria had spread to intercellular spaces and within lysed plant cells inneighboring regions of epidermal, cortical, and vascular root tissues. By 21 dpi, some fluorescent rhizobia had disseminated upward to aerenchyma and vascular tissue within leaf sheaths above the stem base and within leaves. GFP-tagged S. meliloti 1021 heavily colonized these above-ground internal tissues by 35 dpi. These observations were similar using the two rice varieties cv. Zhonghua 8 and cv Nipponbare.

The ability of the gfp-tagged rhizobia to influence the vegetative growth, photosynthetic activity, and reproductive capacity of rice was assessed on plants grown outdoors in potted soil under ambient conditions. All of the plants inoculated with certain strains grew better than did the uninoculated control plants. Among the five test strains, Azorhizobium caulinodans ORS 571, Sinorhizobium meliloti 1021, and Mesorhizobium huakui 93 enhanced rice growth more than did the other two test strains (Table ? 2). Consistent with previous studies, inoculation of rice with these test strains of gfp-tagged rhizobia evoked positive growth responses such as significantly increased root volume, shoot dry weight, shoot height, shoot N content, and grain yield compared to uninoculated control plants (Table ? 2).

Likewise, rice plants inoculated with certain test strains had significantly increased surface areas of flag leaves (considered to have the highest photosynthetic efficiency), net photosynthetic rate, stomatal conductance, transpiration velocity, and water utilization efficiency (Table ? 3) indicating that rhizobial inoculation of rice can also evoke physiological responses resulting in increased photosynthetic capacity and resistance to drought, even when the bacteria cannot be cultured from within these same plant tissues. Influence of rhizobial inoculation on growth physiology of rice: The ability of the gfp-tagged rhizobia to influence the vegetative growth, photosynthetic activity, and reproductive capacity of rice was assessed on plants grown outdoors in potted soil under ambient conditions. All of the plants inoculated with certain strains grew better than did the uninoculated control plants.

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1. 28 ± 0. 08AB | 51 ± 4C | aData are represented as means per pot from three pot replicates, each containing 15 transplanted plants per strain.

Values followed by a different superscript capital letter are significantly different at the 95% confidence level according to Duncan's multiple-range test. Growth responses of potted rice plants 160 days after inoculation with various gfp-tagged strains of wild-type rhizobia TABLE 3 | Rhizobiumtest | Net | Stomatal | CO2 concn (? mol| Transpiration | Water utilization | Area (cm2) | Length/width of| | strain | photosynthetic | conductance | mol? 1) within | velocity (? mol | efficiency (net | of flag leaf| flag leaves ± | | | rate (? mol of |(? mol of H2O | intercellular | of H2O m? 2 s? 1) | photosynthetic |(S = 0. 75 ? | SE | | | CO2m? 2 s?) ± SE | m? 2 s? 1) ± SE | leaf spaces ± |± SE | rate/transpiration | length ? | | | | | | SE | | velocity) ± SE | width) ± SE | | | Ac-ORS571 | 16. 4223 ± 1. 3903A| 0. 2402 ± | 231. 5825 ± | 4. 5299 ± 0. 3397A| 3. 6264 ± 0. 1715BC | 17. 6437 ± | 14. 8490 ± | | | | 0. 0225A | 6. 8692B | | | 4. 9422ABC | 3. 0377D | | Sm-1021 | 14. 9850 ± 1. 6388B| 0. 2180 ± | 221. 3119 ± | 3. 7224 ± | 4. 0222 ± 0. 1922AB | 20. 250 ± | 16. 1600 ± | | | | 0. 0374AB | 11. 5020B | 0. 3272BC | | 3. 9161A | 1. 6222CD | | Sm-1002 | 13. 7036 ± 0. 7259B| 0. 1941 ± | 225. 3235 ± | 3. 3079 ± 0. 2186C| 4. 1549 ± 0. 3220A | 19. 5789 ± | 18. 3021 ± | | | | 0. 0173B | 19. 0891B | | | 4. 4695AB | 2. 0242B | | Rl-2370 | 13. 8499 ± 0. 3768B| 0. 2408 ± | 261. 7970 ± | 4. 1640 ± | 3. 3584 ± 0. 4119C | 18. 9789 ± | 17. 6614 ± | | | | 0. 0403A | 15. 635A | 0. 4180AB | | 4. 4902AB | 2. 6436BC | | Mh-93 | 13. 8633 ± 0. 7615B| 0. 2187 ± | 252. 8500 ± | 4. 3762 ± | 3. 1778 ± 0. 2521CD | 16. 7857 ± | 16. 4579 ± | | | | 0. 0169AB | 8. 0899A | 0. 2911AB | | 3. 4302BC | 2. 6239CD | | Control | 10. 2302 ± 1. 0323C| 0. 1899 ± | 262. 6345 ± | 3. 8537

± | 2. 7688 ± 0. 6929D | 15. 2362 ± | 21. 1538 ± | | | | 0. 0324B | 24. 6411A | 0. 9159BC | | 4. 0063C | 2. 1794A | Data are represented as means per pot from three pot replicates, each containing 15 transplanted plants per strain. Values followed by a different superscript capital letter are significantly different at the 95% confidence level according to Duncan's multiple-range test. Parameters of photosynthetic rate, stomatal conductance, CO_2concentration, transpiration velocity, water utilization efficiency, and morphological change in flag leaves. These results extend the list of benefits to rice after inoculation of their rhizospheres with certain strains of rhizobia by further revealing various additional indexes of growth physiology that are enhanced by this endophytic association.

We plan to conduct additional studies (e. g. , in situ host gene expression activated by bacterial quorum sensing cell-to-cellcommunicationevents plus proteomic/metabolomic analysis of the plant-microbe interactions) to explain why the magnitude of plant growth responses to some inoculant test strains varied significantly.

Conclusion

It is clear from this and earlier studies that rhizobia possess traits that allow them to associate beneficially with a wide diversity of host plants, not only in the classic legume root-nodule symbiosis but also in the endophytic association with cereal plants.

This study shows that various species and strains of rhizobia can not only use “ crack entry” at lateral root emergence as the means to gain access into and colonize the interior of rice roots but also utilize a dynamic infection

process that permits them to migrate endophytically upward into the stem base, leaf base, leaf sheaths, and some leaves of the plant. There they grow transiently to high local populations that are metabolically active, and some rhizobia persist throughout the vegetative and into the reproductive phases of development. Such intimate interactions elevate phytohormone levels within the rice tissues, and also a variety of beneficial responses ensue, impacting significantly on the growth physiology of the rice plant.

These new findings indicate that the natural, endophytic Rhizobium-rice association is far more complex, inclusive, dynamic, and invasive than previously thought, therefore heightening its status as an exciting experimental research model of beneficial plant-bacterium interactions and its potential value for future exploitation as a biofertilizer strategy in sustainable agriculture to produce the world's most important cereal crops for human nutrition.

Reference____ 1. Rice Bradyrhizobium Rhizosphere Gnotobiotic 20 (total biomass) 2. Chaintruel et al. (2000); 3. Bhattacharjee et al. (2008). 4. Yanni et al. (1997) 5. Biswas (1998) 6. http://www. ncbi. nlm. nih. gov