

RECENT STUDIES ON THE *RHIZOBIUM*-CEREAL ASSOCIATION

Frank B. Dazzo¹, Youssef G. Yanni², Rizk Rizk², M. Zidan², Abu-Bakr M. Gomaa³, Andrea Squartini⁴, Yu-Xiang Jing⁵, Feng Chi⁵, and Shi-Hua Shen⁵

¹Dept. Microbiology, Mich. State Univ., East Lansing, MI USA; ²Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt; ³Agric. Microbiol. Dept., Nat. Res. Centre, Cairo, Egypt; ⁴Dipt. Biotechnologie Agrarie, Univ. Padova, Padova, Italy; ⁵Inst. Botany, Chinese Acad. Sciences, Beijing, China

Studies conducted worldwide have validated the natural, endophytic association of rhizobia with cereals (e.g., rice, wheat, barley, wild rice, maize, sorghum, millet) in rotation with legumes, and its strain / variety specificity in promoting vegetative growth, grain yield and agronomic fertilizer N-use efficiency. *Rhizobium leguminosarum* bv. trifolii is the dominant species of rhizobia capable of forming an endophytic association with rice and wheat in the Egyptian Nile delta. A total of 21 out of 23 field inoculation trials conducted so far in the Nile delta have indicated that these cereal crops can benefit from inoculation with selected rhizobial endophytes, based on their direct ability to promote cereal growth independent of BNF, plus the newly fixed nitrogen provided by BNF with the clover rotation. The natural spatial distribution of a high-performing, biofertilizer-candidate strain indigenous to the Nile delta is being mapped by a combination of microscopy, image analysis, and geostatistics in crop fields at the Km-scale relevant to the rice farmer and on rice roots at the μm scale relevant to the microbe. For these geostatistical studies, new indices of local abundance have been introduced as the *Z* variate for both spatial scales over the sampled domains.

Other related studies have been done to gain a better understanding of the route(s) used by rhizobia to enter, disseminate and colonize the interior of rice plants. For these studies, a plasmid conferring stable, constitutive expression of *gfp* and antibiotic resistance markers was introduced into various wildtype rhizobia (*Sinorhizobium meliloti*, *R. leguminosarum* bv. viciae, *Azorhizobium caulinodans*, *Mesorhizobium hankuii*). Populations of these marked rhizobia associated with various rice tissues were analyzed by a combination of fluorescence confocal microscopy, CMEIAS *in situ* image analysis, and viable plate counts on media containing appropriate antibiotics. The results indicated a dynamic infection process in rice, beginning with preferential colonization and entry of the bacteria at lateral root emergence, followed by their endophytic ascending migration into and growth within aerial plant tissues that include the stem base, leaf sheath and leaves of rice. Thus, the endophytic rhizobia-cereal association should be considered as far more invasive than previously thought, therefore heightening its interest as an experimental research

model of plant-microbe association and its potential value for exploitation in sustainable agriculture to produce the world's most important cereal crops for the future.