Tools Towards Managing Mycorrhiza for Sustainable Agriculture

The arbuscular mycorrhizal (AM) fungi, in concert with other soil dwelling microorganisms such as the so-called “plant growth promoting rhizobacteria”, play a pivotal role for natural soil fertility and stability, hence, for sustainable crop production. Appropriate management of the AM fungal community is crucial, especially in the tropics where the cultivated soils are notoriously fragile and prone to degradation. The discovery of the multiple benefits of the AM symbiosis provided to the crops induced a global mushrooming of enterprises commercializing AM fungal inoculants to be used as “bio-fertilizers”. A serious drawback of these endeavors was that the AM fungal inoculants could not be identified at the strain level, precluding a scientifically rigorous test of the origin and persistent quality of such inoculants. Furthermore, after field application the inoculated AM fungal strains could not be traced precluding verification of root colonization by the strains in target crops and studies on the strain competitiveness and persistence in the field amidst the native AM fungal community. The “holy grail” for researchers on AM fungal ecology and application was therefore to obtain tools allowing specific tracing of AM fungi at the strain level in soil and roots sampled from field sites. We are happy to report that two of our approaches to attain this goal were successful. We also accomplished our additional tasks, namely to assess the native AM fungal communities present at the various experimental field sites in India prior to the application of the AM fungal “bio-fertilizers” and, furthermore, to develop experimental test systems at the laboratory scale for screening important functional traits of isolated AM fungal strains.

Task 1: Assessment of the native AM fungal communities at SA-ISCB field sites

This task was accomplished in collaboration with Dr. Anil Sharma (GBPUAT). At the nine SA-ISCB experimental field sites, soil samples were collected prior to the application of the AM fungal “biofertilizers”. The AM fungal spores were isolated, identified on the basis of spore morphology and counted to assess the AM fungal community structure. In total, 38 AM fungal species were detected, belonging to 18 genera and 7 families.

Task 2: Development of functionality test systems for screening isolated AM fungal strains

Test systems were developed to assess the following important functional traits of AM fungi: (i) rate of spreading of the AM fungal hyphae in the soil and, concomitantly, rate of propagation of AM root colonization among neighboring plants, (ii) AM fungal efficacy of nutrient supply to model plants and (iii), using an experimental mixed-cropping system with *Sorghum* and *Linum*, the return of carbon investments by the two crops for the buildup of a common mycorrhizal network in the soil, in terms of nutrient gains (P, N) by the crops via this mycorrhizal network. AM fungal hyphae were found to spread and colonize neighboring plants at rates between 1.4 to 4.0 mm per day. Remarkably, in the mixed crop experiment it was found that *Sorghum* supplied about 80% of the carbon invested in the common AM-hyphal network but received only about 20% of the P and N provided to the two crops via the network. Thus, *Linum* substantially profited from a neighbouring sorghum in the presence of AM fungi. Overall, the mixed-cropping system turned out to be appreciably more productive than...
the mono-cropping control systems, supporting traditional agricultural practices that used to take advantage from mutual facilitation of crops obtained in appropriate mixed-cropping systems.

Task 3: Development of strain specific markers for tracing AM fungi

Our main task, to develop molecular tools for AM fungal identification and tracking at the strain level was particularly risky because of the peculiar and still poorly understood genetics of AM fungi. For instance, each AM fungal spore generally possesses numerous variants of the highly variable “ITS” region of rDNA, which is the region commonly used for identification. Thus, different strains or isolates within a species, and even closely related species, cannot be distinguished by using the rDNA region. We chose two novel approaches for AM fungi, namely a search for markers based on the mitochondrial rDNA and on microsatellite loci and, luckily, found the following markers for strain specific tracing: (1) eighteen polymorphic microsatellite markers for *Glomus intraradices*, which is the worldwide most widely distributed and most frequently applied AM fungal species; up to six alleles per locus (average four) were found in eight randomly selected *G. intraradices* strains (see Appendix). (2) Mitochondrial markers based on the large subunit rDNA region. In particular, a set of highly specific primers was developed for tracing the *G. intraradices* strain “TERI commercial”. Unlike in the case of the nuclear rDNA, no intrasporal variation occurred with our novel markers (1) and (2). Both markers are the first of their kind for AM fungi and have an immense potential for basic and applied research. We have tested them already in pot cultures in the presence of a native AM fungal community, where we could detect the introduced strains specifically in samples of colonized roots. We are confident, therefore, that the tools will be successful also with root samples from field experiments for tracking of introduced strains.