**Portfolio · First Phase (1999-2004)**

**Introduction of Arbuscular-Mycorrhizal Fungi (AMF) as Bio-Fertiliser and Soil-Structure Stabiliser for Sustainable Agriculture**

**Project ID:** SA6

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**RATIONALE**

Mycorrhiza literally means 'fungal-root' and refers to an intimate, mutually beneficial symbiotic association between plant roots and certain fungi. It is estimated that more than 80% of higher plant species interact with specific fungi among which the **arbuscular mycorrhizal fungi** (currently grouped to the Zygomycetes, order of Glomales) show the broadest distribution. The mycorrhizal symbiosis offers several benefits to the plants: improved nutrient uptake, faster growth, greater drought resistance, protection from pathogens, increased seedling survivorship, improved soil structure and greater resistance to invasion by weeds.
The most thoroughly studied benefit of the mycorrhizal association is the growth stimulation of the host plant due to improved phosphorus nutrition. This is of particular importance for India where nearly half of the districts are classified as highly deficient in plant available phosphorus. Moreover, the uptake of nitrogen and micronutrients such as copper and zinc can be increased significantly.

Although the beneficial effects of **arbuscular mycorrhizal fungi (AMF)** on their host plants, especially in poor soils, have been known for a long time, many fundamental questions concerning this symbiosis and its management are still unsolved. Based on this situation, we will use a combination of classical and molecular methods in order to elucidate the major factors determining the beneficial interactions between AMF and important crops in India, particularly wheat and pulses. Our final goal is to apply AMF as biofertilizers in crop production in order to allow a reduced input of chemical fertilisers and to achieve an increased sustainability in agriculture.

The following research activities are planned within the frame of this ISCB project:

- Isolation and propagation of indigenous AMF communities from selected natural habitats as well as from low-input and high-input agricultural sites in India and Switzerland; characterisation of the isolated AMF communities
- Production of single-spore derived AMF cultures and axenic, in vitro cultures of AMF on Agrobacterium rhizogenes transformed root organ cultures (ROC’s)
- Testing the efficiency of AMF isolates with respect to plant growth promotion under conditions of low nutrient supply; comparing the growth promoting capability of the most efficient AMF isolates with different Indian wheat varieties
- Testing AMF isolates for their capacity to transfer nitrogen from legumes to wheat crops
- Studying the interactions of the mycorrhizal symbiosis with plant growth promoting rhizobacteria (PGPR) and other soil microorganisms
- Propagation and re-introduction of the most promising AMF isolates in agricultural production systems

So far, numerous AMF communities from various sites in India and Switzerland have been isolated and propagated in trap cultures. The diversity of these isolates has been determined by identifying AMF species using the taxonomy based on the morphological features of the AMF spores. These studies indicate that high abundance and diversity of AMF is favoured by natural or low-input agricultural sites, raised bed plantations in irrigated fields and by soil management systems which do not disturb the physical soil properties (no tillage systems).

Preliminary studies on single-spore derived AMF cultures and on AMF in vitro cultures growing on ROC’s were recently initiated.
SUMMARY OF THE ACHIEVEMENTS OF THE FIRST PROGRAM PHASE
adapted from the summaries provided by the project partners

Indian Partner (Dr Adholeya):
The modern, intensive agricultural practices are currently being re-evaluated and are coming under increased scrutiny as the awareness of the consequences of excessive use of fertilizers and chemical pesticide usage improves. The concept of biofertilizers is to domesticate some of these microorganisms in agricultural production systems, so that the vast natural reservoir of nutrients in the atmosphere can be tapped as an additional source to meet the requirements of sustainable agriculture. It also augments yields and monetary returns to the farmers, particularly to the small and marginal ones, for which the incremental input cost is low. A mycorrhizal biofertilizer for wheat has been screened and selected by the Centre for Mycorrhizal Research (CMR) of The Energy and Resources Institute (TERI) in the SA6 project.

TERI executed the project in three phases. The first phase of the project primarily aimed at collecting samples from different agro-climatic regions of
India where wheat-rice rotation is in practice, enumerating diversity of arbuscular mycorrhiza fungal (AMF) isolates, analyzing soil nutritional properties, and identification of sites for collecting AMF for raising trap cultures for the next phase of the project. Although these fungi have extremely wide host ranges, they are very specific in their effects on plant species. Thus, it becomes necessary to have a repository of these fungi from different agro-climatic regions. The collection of strains in the repository forms the basis for functional tests. For this purpose, a total of 139 trap cultures were raised from 6 different regions. Three successive trap culture cycles were maintained using different host plants, *Allium cepa* (onion), *Tagetus spp.* (marigold), *Daucus carotus* (carrot), *Medicago sativa* (alfalfa), *Trifolium alexandrium* (berseem) *Gossypium spp.* (Cotton), *Vetiveria zizanioides* (Vetiver), *Vigna radiate* L. Wilezek (mungbean), and *Sorghum spp.*. The spores from the trap cultures were used to raise monospecific cultures (from both polyspores and single-spores). Out of 320 cultures raised, a total of 186 pure monospecific cultures were established successfully (60 from single-spores and 126 polysporals) after three culture cycles. Two new species were grown successfully under *in vitro*. Understanding of these two new isolates towards functional response was conducted under *in vitro*. Optimization of the growing pattern of these new cultures was also performed successfully using different media manipulations. Co-culture experiments using these new isolates along with already existing *Glomus intraradices* and *Gigaspora margarita* were conducted with the aim to establish an *in vitro* consortium. Tobacco-, tomato-, raspberry-, cotton-, mung bean-, and marigold-roots were transformed to establish root cultures. The optimization of root cultures is underway. Co-culture experiments of AM fungi *Glomus intraradices* on Ri T-DNA transformed clover roots along with several PGPR’s bacterial cultures procured from Neuchatel group was jointly carried out *in vitro* with the Neuchatel group.

In the second phase, functional performance of AMF-communities from trap cultures was studied in greenhouse experiments. AMF-communities were first characterized on the basis of nitrogen (N), phosphorus (P), potassium (K) and organic carbon (OC) uptake in the above-listed host plants of trap culture cycles. AMF spore count, intraradical AMF colonization and infectious propagules were also noted. The responses of host species w.r.t. dry matter, NPK and OC content in the shoot varied with the isolate. The isolates of the same region also had variable responses on the same host. Then, AMF-communities of trap cultures were characterized on the basis of growth and nutrient uptake of wheat. In another experiment, AMF-communities from the Chambal region (natural, undisturbed region) were inoculated on wheat (UP 2338 variety). Different dosages of AMF-communities were given to the wheat plants and the plants were supplied with two levels of Hoaglands’ nutrient solution (H, half dose; F, full dose). These communities were characterized on the basis of growth promotion and nutrient uptake of wheat plants. From these functionality experiments, the four most efficient AMF-communities were selected for further testing of their efficacy.

In the third phase of the project, one *in vitro* based AMF culture and the four most efficient communities selected were further tested in greenhouse as well as in the field. A greenhouse experiment was set up using these communities to test their symbiotic performance with modern, high yielding wheat cultivars, ancient landraces and wild relatives of wheat (a total of 13 varieties: 6 modern hexaploid wheat varieties of *Triticum aestivum* i.e. UP 2338, HW 2004, HD 2687, PBW 343, HW 2045 and Kundan; 2 tetraploid wheats i.e. *T. dicoccoides* and *T. turgidum* and 5 diploid wheats i.e. *Aegilops squarrosa*, *A. speltoides*, *A. sharonensis*, *T. uratu* and *T. monococcum*). These AMF isolates were also tested for their response on wheat when supplied together with rhizobacteria (11 PGPRs which were provided by the Pantnagar group). A microcosm experiment was done to study the role of interaction between these AMF and PGPRs with respect to mycorrhiza formation, wheat growth response, and plant nutrient acquisition. Field studies were conducted in five different agro-climatic regions to evaluate whether the five selected AMF are functionally superior for the enhanced production of wheat. The selected AMF isolates were tested under different cultivation systems (conventional, zero-tillage and raised-bed sytems) and under different input conditions (with various combinations of fertilizer,
manures and under organic conditions. Up to 50% savings on inorganic fertilizers was achieved, while improving the yield of wheat in the field by 10%.

Other activities included a training for Mr K. Ineichen from Basel at TERI, visits of project partners in India and Switzerland, as well as the organisation of a training workshop.

**Swiss partner (Dr Wiemken):**
The primary objectives of the Swiss part were (i) to investigate the impact of different use systems on the genetic and functional diversity of Arbuscular Mycorrhizal Fungi (AMF) and, building on the outcome of these studies, (ii) to select and propagate AMF strains exhibiting outstanding efficacy in plant growth promotion and soil structure stabilization. The project was linked with the SA7-project, which was focused on rhizobacteria, specifically on the so called “Plant Growth Promoting Bacteria” (PGPR), aiming at the same overall goal: to develop microbial resources enhancing soil fertility as a prerequisite for obtaining low-input, satisfactory and sustainable crop production. Both projects were focused on wheat, but other crops were also considered, particularly legumes because of their paramount importance in agroecology; cereal/legume intercropping was common practice in the past, is still applied in vast semi-arid regions of India with marginal rain-fed agriculture, and is a topic again in the strive for developing more sustainable forms of agriculture, particularly in the tropics.

In a first step, the abundance and genetic diversity of AMF were studied in agro-ecosystems widely differing in land use intensity. The AMF were identified by the classical method of morphotyping the spores. Spores were isolated directly from field soil samples and, additionally, from AMF-trap cultures set up by using samples of field soil for inoculating a set of suitable AMF host plants. Molecular tools allowing an identification of AMF in root samples were developed (Redecker et al 2003). Using these tools, species from several AMF genera (*Glomus, Acaulospora, Scutellospora, Paraglomus* and *Archeospora*) were detected in roots sampled from the different agro-ecosystems as well as from the trap cultures. Both the morphological and molecular approaches correspondingly revealed that intensive high-input farming applying continuous mono-cropping is deleterious for AMF diversity. However, contrary to previous claims, it was found that AMF diversity is not necessarily low in arable soils, provided that an ecologically sound farming is practiced, including appropriate crop rotations and cover crops. Results from these agro-ecological studies were published (Oehl et al., 2004; Oehl et al., 2005).

In a second step, AMF functional diversity was assessed. To this end, a large collection of AMF strains derived from the divers agro-ecosystems investigated had to be built up. The collection presently held at the Botanical Institute in Basel (BIB) comprises about 400 monospecific AMF strains all derived from single spores. So far, only 40 of these strains could be examined in more detail by functional tests in association with wheat and some other crops. Nine strains proved to be especially beneficial in various functional tests that were conducted at BIB and the Research Institute for Organic Agriculture in Frick (FIBL). Some of these strains were used also for studies conducted in collaboration with the project partners SA6/7 from TERI, the University of Neuchatel and Pantnagar University. It was found that the effects of AMFs on plants are highly variable ranging from beneficial to slightly detrimental depending on the specific combinations between AMF strains and crop plants. This showed that a selection of AMF strains exhibiting outstanding beneficial functional traits is feasible. In functional tests conducted in the greenhouse, strains of the following species enhanced grain yields of both wheat and soybean most efficiently: *Glomus mosseae*, *G. lamellosum*, a “G. species resembling G. etunicatum”, *G. aureum* and *G. constrictum*. Strains of *G. aureum* and *G. constrictum* were found especially efficient in a long term experiment where, after an AMF-inoculated pre-crop of soybean, the grain yield of succeeding wheat was measured. Remarkably, the AMF strains found to efficiently promote crop performance in our functional tests were originating almost exclusively from low-input agro-ecosystems.
The scientific exchanges of researchers between partners were undoubtedly the highlights in the joint project SA6/SA7, leading to tremendous knowledge transfer and capacity building. Specifically, by organizing several highly informative field trips with visits of farms and agricultural research stations, the Indian partners introduced the Swiss partners to the manifold agroecosystems occurring in Northern India. The Swiss partners thus gained invaluable insights into the great potentials – as well as problems – of agriculture in India. Conversely, the Swiss partners introduced various advanced research methods to the Indian partners. Considerable knowledge transfer could take place during the short stay of Mr Kurt Ineichen at TERI, during the extended stays of Ms P. Tiwari (TERI), Dr A. Sharma and Ms D. Dwivedi (University Pantnagar) at BIB, and of Mr G. Reddy (TERI) at FIBL. Moreover, several researchers from TERI and Pantnagar shortly visited BIB during a stay at the University of Neuchâtel. In conclusion, the opportunity to organize mutual scientific visits and extended stays of motivated researchers in the partner laboratories were the most rewarding activities in this ISCB initiative, with respect to the knowledge transfer, capacity building and last not least, new friendships and mutual cultural understanding.

Publications:
