▶ *Keynote presentations*

K-1139 Molecular approaches to rhizosphere research: identification and characterization of aluminum tolerance genes and their use to improve acid soil tolerance

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Aluminum (Al) toxicity is a major limiting factor to agriculture on acid soils that make up approximately 30% of the world's total land area and up to 50% of the world's potentially arable lands. A large proportion of the acid soils occur in developing countries in the tropics and subtropics; thus, Al toxicity reduces food security in many parts of the world where it is most tenuous. Because of the agronomic importance of crop Al toxicity, identifying the molecular determinants for Al tolerance has attracted significant interest from a number of laboratories around the world. We are now poised, based on recent discoveries by a number of researchers, to develop the molecular and genetic resources required to address a worldwide agronomic problem that is only exceeded by drought stress with regards to abiotic limitations to crop production.

In this presentation, the molecular approaches that have been used to identify Al tolerance genes in wheat, *Arabidopsis* and sorghum will be discussed. For example, in sorghum, using high-resolution mapping, we identified AltSB as a candidate Al tolerance gene and subsequently verified its role in Al tolerance based on: 1) a strong correlation between Al-inducible AltSB expression and Al-inducible tolerance and root citrate exudation; 2) a member of a gene family involved in the efflux of organic solutes; 3) protein localization to the plasma membrane; and 4) AltSB expression in transgenic *Arabidopsis* resulting in significant increases in Al tolerance and Al-activated root citrate efflux. Sequence analysis of the parental AltSB alleles and the promoter region from selected members of a sorghum diversity panel, combined with determination of AltSB expression, Al tolerance and root citrate exudation indicates that differences in gene expression are a major determinant of differential tolerance in sorghum. Furthermore, a MITE-type transposable element in the AltSB promoter appears to be a highly polymorphic motif in the promoter region and may play a role in the differential Al-inducible expression.

K-1094 Burkholderia diazotrophic species exhibit mechanisms involved in plant growth promotion, biological control and bioremediation

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Burkholderia species, particularly those grouped in the *Burkholderia cepacia* complex (Bcc), constitute one of the dominant bacterial groups in the maize rhizosphere. However, Bcc bacteria have been found in high prevalence as opportunistic pathogens in cystic fibrosis patients. This fact has hampered the commercial development of some Bcc strains in biotechnological processes. In contrast, studies performed on the association of *Burkholderia* species, different to the Bcc, with important agricultural plants are limited. For a long time, N_2-fixing ability in *Burkholderia* species was recognized only in B. vietnamiensis, a member of the Bcc. Novel rhizospheric N_2-fixing *Burkholderia* species associated with plants have been described recently, e.g., *B. unamae*, *B. xenovorans*, *B. tropica*, and *B. silvatlantica*. These diazotrophic *Burkholderia* are closely related, but phylogenetically largely distant from the Bcc species. In fact, cblA and esmR genes encoding transmissibility factors identified among clinical strains from the Bcc were not detected in many strains of *B. unamae*, *B. tropica* or *B. silvatlantica*.

Apparently, the N_2-fixing *Burkholderia* have a large capability of association with plants, as they have been isolated from the rhizosphere of maize, sugarcane, sorghum, coffee, and tomato. The widespread association of such N_2-fixing *Burkholderia* with plants could represent new opportunities in biotechnological processes. Although the involved mechanism is unknown, *B. unamae* and *B. tropica* improve the maize plant growth. *B. tropica* exhibit a great mineral phosphate-dissolving capability, and *B. unamae* exhibit high ACC deaminase activity. In fact, the ACC deaminase activity is a common trait among the N_2-fixing *Burkholderia* species. In addition, the siderophores production by the diazotrophic *Burkholderia* could represent an important trait for controlling plant pathogens. In the N_2-fixing *Burkholderia* species is also remarkable the capability for using as carbon sources some environmental pollutants, for example, *B. unamae* is able to use phenol and benzene for its growth. This feature and the capability of *B. unamae* for promoting growth of maize and to colonize the rhizosphere and rhizoplane of plants could be suitable in rhizoremediation. Based on the data described, we considered that N_2-fixing *Burkholderia* consortia could be used for promoting plant growth and increasing crop yields, and concomitantly in bioremediation, and biocontrol of plant pathogens.

Oral presentations

O-572 Life in soil: the impact of GM plants on rhizosphere communities

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The worldwide commercialisation of transgenic plants has intensified the public debate on risks and benefits of genetic engineering. If GM plants do affect the highly complex soil-ecosystems, how can these impacts – beneficial or deleterious – be assessed? Because it is not feasible to monitor all compounds of a soil ecosystem for their response to a GM plant, the use of keystone indicators is required. The most likely habitat to be affected by the transgene expression is the rhizosphere, known as the volume of soil that is influenced biologically and biochemically by the living root. In recent years some keystone indicators have been defined. These include plant-growth-promoting rhizobacteria, nitrogen fixing and nitrifying bacteria, lignin-decomposing fungi, and mycorrhizal fungi. In some cases, the type of GM plant will influence the choice towards more specific keystone indicators.

During the last decade many important baseline data about the shifts of microbial communities in the rhizosphere have been provided to relate potential changes to natural fluctuations, growth stages, cultivars or soil management. It has been shown that the effects of GM plants on root-associated microbial communities are subject to seasonal variation. This presentation will summarise the results of two field studies including an annual GM crop plant and GM trees.

O-740 Effect of rhizosphere bacteria on Zn/Cd mobilisation from a contaminated soil and accumulation of Zn/Cd in metal-accumulating Salix caprea

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Rhizosphere bacteria are considered to play a key role for the plant availability of Zn and Cd in contaminated soils. The bacteria tested in this study were isolated from S. *caprea* rhizosphere (soil) and endosphere (xylem sap, leaf material) obtained from the polluted site Arnoldstein (South Austria). The selected microbes (32 strains) were grown in a tryptic soy broth (TSB) medium for a period of 1 week. Experiment 1: The medium solutions containing the microbes were centrifugated and passed through a 0.45 µm filter. 5 ml of the filtrates were used to extract Zn and Cd from a contaminated soil (1 g) derived from Arnoldstein. After 2 h extraction on an end-over-end shaker (20 rpm), the samples were centrifugated and passed through a 0.45 µm filter. The final filtrates were analysed for Zn and Cd. Experiment 2: Roots of one year old *S. caprea* plantlets were soaked in the bacterial suspensions of similar cell density for 30 minutes and subsequently planted in 1 kg aliquots of γ sterilized Zn/Cd/Pb contaminated soil (Arnoldstein) and watered with 12.5 ml of the bacterial suspensions. After 12 weeks of incubation in a growth chamber, leaves were harvested and analysed for total dry weight and for Zn and Cd concentration.

The majority of the tested strains caused an immobilization of Zn and Cd in Experiment 1. Compared to the control (no bacteria), the largest amounts of Zn and Cd could be mobilized by the filtrates of an endophyte from the Microbacteriaceae familiy and by the rhizosphere bacterium *Agromyces* sp. In Experiment 2, for most strains, no difference was found to the non-inoculated control regrading leaf biomass and foliar Zn and Cd concentration. One strain (*Streptomyces* sp.) caused a significant increase of Zn and Cd concentration in leaves, while another strain (*Agromyces* sp.) induced a signficant effect on leaf biomass. Regarding the total amount of Zn and Cd in leaves, the increase of leaf biomass had a more pronounced effect than the enhanced foliar Zn and Cd concentration.

O-695 Application and plant growth promoting effect of a commercially used biological plant stimulator in barley

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The ability of microbes to stimulate and promote plant growth is of scientific interest since many years. Such microbes were applied to increase agricultural productivity of crop plants. These beneficial bacteria colonise the plant roots and stimulate the plant growth by several mechanisms like phytohormon production or supplying the plant with more nutrients. In this study we analyse the effect of a commercially available biological plant stimulator (Proradix®) after application to barley plants. Our experiments were carried out in lab as well as in greenhouse and field experiments. We also analyse the changes in the bacterial community after the use of Proradix® with molecular methods. To detect the biological active compound in Proradix®, a *Pseudomonas* strain, and to study the colonisation behaviour we use the fluorescence in situ hybridisation (FISH) method with specific oligonucleotide probes in combination with confocal laser scanning microscopy. We also use the gfp tagged *Pseudomonas* strain for this purpose.

We could find the *Pseudomonas* strain in all parts of the root especially in the root hair zone as well as on root tips and endophytic in root cortical cells. Beneficial plant growth promoting effects could be shown. We detected higher yields up to 19% for barley plants treated with Proradix® in field experiments in 2006. The dry weight of shoots and the number of ears were about 44% higher than in the control in greenhouse experiments.

Furthermore the question arises to which extent this *Pseudomonas* strain influences the composition of the rhizosphere bacterial community. For this purpose, terminal restriction fragment length polymorphism analysis is applied and clone libraries are constructed.

O-384 Biological nitrification inhibition: a novel plant-soil interaction for improving wheat production?

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Biological oxidation of ammonia to nitrate is known as nitrification. Ammonia is first oxidised to nitrite by *Nitrosomonas*, and nitrite is further oxidised to nitrate by *Nitrobacter* chemoautotrophic bacteria. Ammonium ions (NH_4^+) are positively charged and bind to the soil exchange complex so rarely leach, thus remain available for crop uptake. However, if ammonium is converted into nitrate (NO_3^-), N can leach out of the rooting zone. Nitrate is also susceptible to denitrification, whereby N is converted and lost from the cropping system in the gaseous forms N_2, NO and NO_2. These gases are partially responsible for global warming. Inhibition of nitrification in agricultural systems would therefore be a considerable gain both economically and environmentally.

Synthetic nitrification inhibitors are commercially available. Some plants, such as the tropical grass *Brachiaria humidicola*, were proposed to release compounds from their roots that suppress nitrification (Ishikawa et al 2003). A highly sensitive bioassay was developed using a recombinant *Nitrosomonas europaea* that can detect nitrification inhibitors released from roots (Iizumi et al 1998; Subbarao et al 2006). The presence of ammonium is the stimulus for release of inhibitors from *B. humidicola* roots (Subbarao et al 2007a). Inter- and intra-specific differences among plant species in the capacity to release inhibitory compounds exist (Subbarao et al 2007b).

Recently *Leymus racemosus*, a wild relative of wheat used by breeders to transfer disease resistance traits into cultivated wheat, was discovered to have the ability to inhibit *N. europaea* function. In contrast, wheat cultivars have not yet been shown to have this ability (Subbarao, unpublished). This discovery opens exciting possibilities for the development of wheat cultivars that have a built-in ability to inhibit nitrification in soils, thus minimising nitrogen losses. We will report on our recent exciting findings about biological nitrification inhibition (BNI) physiology, release of BNI compounds, and variation in BNI capacity among wheat cultivars, landraces and wheat-*L. racemosus* hybrids.

Ishikawa T. et al 2003 Plant and Soil 255:413-419
Iizumi T. et al 1998 Applied and Environmental Microbiology 64:3656-3662
Subbarao GV. et al 2006 Plant and Soil 288:101-112
Subbarao GV. et al 2007a Plant and Soil 290:245-257
Subbarao GV. et al 2007b Plant and Soil (in press)

O-844 Introducing deep rooted plant species for improved nitrogen management in agriculture

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A main problem in agricultural nitrogen (N) management is that surplus N is leached down through the soil and is eventually lost to the environment. One solution to this is to grow catch crops during the autumn, when precipitation surplus is high and the soil would otherwise be left uncropped (Thorup-Kristensen et al., 2003). Previous studies have shown that fast and deep root growth by catch crops is important for reducing N leaching losses. In many areas, catch crops are established together with a cereal crop, but mainly develop and cover the soil in the autumn after cereal harvest. Currently, farmers mainly use grasses for this purpose, but grasses are slow to develop deep root systems.

In this study, we have tried to find deep rooted alternatives to the grasses. We have studied almost 20 plant species from different plant families, most of them not currently grown in agriculture. We have studied their development, root growth, final rooting depth and ability to take up nitrogen from deep soil layers down to 2.5 m depth. The results show large differences among the species in root development during the autumn. Ryegrass and some other species developed roots only to 0.75 to 1 m depth in the soil, whereas the two most deep rooted species were able to develop high root densities to at least 2.4 m depth. Results of soil analysis in November showed that all species were efficient in depleting the top 1 m of the soil, but in soil layers below 1 m, there was a clear relationship between rooting depth and depletion of the soil inorganic N content. The most promising species was *Isatis tinctoria*, but other promising species were identified as well. It can be concluded, that by studying wild plant species with different adaptations and root growth patterns, we can find tools to help farmers to develop more N efficient cropping systems and to reduce N losses to the environment.

Thorup-Kristensen, K. (2006) Effect of deep and shallow root systems on the dynamics of soil inorganic N during three year crop rotations. Plant & Soil 288, 233-248

Thorup-Kristensen, K., Magid, J. & Jensen, L.S. (2003) Catch crops and green manures as biological tools in nitrogen management in temperate zones. Adv. Agron. 79, 227-301

Poster presentations

P-737 Selecting tomato (*Solanum lycopersicon* L.) lines for mycorrhizal competence: a pre-requisite for breeding the plants for the future

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Recent breeding programs revealed that it is difficult to select varieties for low-input agriculture by starting from conventional varieties. One of the explanations given for this failing is that conventional varieties, selected in (under) high-input environments (conditions), would have lost genes coding for symbiotic competence. For example, conventional varieties generally show a lower capacity to sustain root symbiosis with naturally occurring arbuscular mycorrhizal fungi (AMF), than needed for low-input agriculture. Such loss in mycorrhizal competence was probably due to breeding in fully fertilized soils, which resulted in the selection for plant genotypes unable to exploit these beneficial microorganisms.

Therefore, the goal of our research was to select, among 10 mutant lines, belonging to a tomato (cv Red Setter) EMS mutant collection, those presenting both a good mycorrhizal competence and a high level of productivity, in order to obtain tomato genotypes adapted to breeding in low-input cropping systems.

The wild type line Red Setter, as well as all the 10 mutated ones, showed a characteristic AMF infection dynamics. Some differences were also found in the diversity of root-colonizing AMF taxa. One tomato mutant line was specially mycorrhizal competent, and showed high precocity and a highly structured symbiotic population. Other mutants were similar to wild type line (control), and more research is needed to clarify their actual position.

P-822 Plant growth promoting rhizobacteria to improve productivity and plant health of potato based cropping systems in the Central Andean Highlands

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Plant Growth Promoting Rhizobacteria (PGPR) have shown their potential in various crops to improve yield and plant health. In this respect PGPR could be developed into an alternative inexpensive bio-fertilizer to increase potato yields of small scale subsistence farmers and/or to improve organic crop production systems in the Central Andean Highlands of Peru.

In 2005/06 commercially available PGPR were applied in field trials with potatoes resulting in yield increases of 20 to 30% and partial control of *Rhizoctonia solani*. Different potato varieties and genotypes showed distinctive response to PGPR in terms of growth promotion, disease control and maintaining bacterial population during the growth period. Yield increases were due to greater number of tuber/plant and also increased tuber weight.

Furthermore, more than 100 PGPR (*Bacillus* spp., *Azotobacter* spp., Actinomycetes.) were collected in different regions of the Central Andes. They were selected and partially identified using laboratory and greenhouse tests such as control of R. *solani*, *Fusarium solani*, production of indoleacetic acid, solubilization of phosphates and their effect on plant growth. In pot trials various of these PGPR increased tuber production two to threefold. Plants inoculated with PGPR had a greater leaf area and a lower specific leaf area (SLA) than non-inoculated plants. SLA is directly correlated with a higher photosynthetic activity. In addition inoculated plants showed an increased uptake of N, P, and K and an altered distribution of N and K within plants. A greater portion of the total N adsorbed was distributed to leaves and tubers and more K was found in tubers as compared to control plants, indicating again an increased photosynthetic activity and a stronger tuber sink (resulting in higher tuber weights).

The best twelve PGPR, combining several of these traits, are presently tested in field trials with potato at three locations (Huancayo, Puno and Aymara, 3800 m above sea level). With the results of these trials we hope to verify pot trial results and develop a protocol for selection of effective PGPR. Best PGPR will then be multiplied for regional trials.

P-1186 Interactions between rhizobias native of Senegal and French bean lines (*Phaseolus vulgaris* L.) contrasting on tolerance of low phosphorus (P) level of soil.

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The snap-bean is the most exported culture from Senegal representing more than 60 % of horticultural exports. Its production is only limited by the poverty of the soils in essential elements (N, P), and by nitrate pollution of the ground water due to the concentration of its culture in the Niayes zone. Thus, the farmers use large quantities of urea to fertilize bean, which is very expensive and aggressive against the environment. The symbiotic nitrogen fixation (SNF) can be used to substitute chemical fertilizers and to reduce the production costs, but it is limited by soil-P deficiency. To circumvent this limitation we propose to use lines with high Efficiency in Use of P (EUP) and high SNF with efficient native rhizobias, and verify their synergestic effect on the plant growth with a reference mycorrhiza. The preliminary results show better yields in term of biomass of shoot, root and nodules of the local snap-bean cultivar with rhizobias ISRA 554 and ISRA 711. However the field inoculation with rhizobias alone does not improve the root-occupancy by mycorhiza. By contrast, the association of rhizobial ISRA 554 and myccorhizal *Glomus mosseae* (Gm) inoculation, with promotes a higher myccorhization than GM inoculation alone. It is prospected to study the effects of the targeted inoculants on the rhizopheric exudation of protons and organic acids by the symbiotic-roots, and their consequence on the bioavailability of soil-P.

P-680 From rice plant to power plant: microbial fuel cells generate electricity from root exudates

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Microbial fuel cells (MFCs) are fuel cells in which bacteria are used to oxidize organic substrate at an anode. This enables direct generation of electrical current using microbial catalysts. Sediment MFCs are a type of MFC in which the anode is embedded in a sediment. This anode is capable to oxidize reduced substrates present in the sediment, with resulting electrical power generation. While these systems thus far mainly focused on marine applications, they may have the potential to be applied in freshwater wetlands and in relation to plants.

Plants release organic compounds into the rhizosphere during growth, and this provides a substrate for microbial root colonizers. Here, we show that these plant derived substrates can be oxidized at an anode. It is hence possible to generate electrical power directly from living plants in a non-destructive way.

To achieve this, sediment MFCs were installed with and without rice plants in a plant support matrix (soil, vermiculite or graphite granules). The anodic potentials were influenced by the release of plant derived reduced materials as well as the release of oxygen through the rice roots. Strong photosynthetic periods with high temperatures were required to mediate the onset of current production from plant derived material. Once the process had started, electrical current densities of up to 38 mA m^{-2} plant growth area (PGA) and average power productions of 17 mW m^{-2} PGA could be attributed to rhizodeposition of the rice plants. As the system was limited by the cathodic counter electrode, higher values are conceivable. Based on theoretical and experimental calculations, 31% of the electron content of the root exudates and 9% of their energy content was harvested as useful power.

Whereas a typical sediment MFC is limited by diffusion to the anode, a living plant can continuously deliver substrate close to the anode, allowing continuous power production and increasing the attainable production from a typical non-planted sediment MFC. The prospect of sunshine driven power generation in (remote) areas with high solar inputs is offered. By oxidizing the prime electron donor for methanogens in the rhizosphere of rice, these MFC systems furthermore open the possibility to diminish methane outputs from wetlands.

P-597 *Pseudomonas aurantiaca*: its role in the rhizosphere of important agronomic cultivars

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The rhizosphere is a unique and complex environment of intense chemical and biological activity. Is defined as the volume of soil around living plant roots that is influenced by root activities: root growth,uptake, respiration and exudation can all directly alter soil properties in the rhizosphere. There were many microorganisms in this region that play important roles in plant development

In this study, the influence of *Pseudomonas aurantiaca* on different plant species (alfalfa, soybean, wheat, sunflower, maize among others) and the population dynamics was investigated.

Pseudomonas aurantiaca is able to colonize different root-systems crops, maintaining an appropiate population in rhizospheric area and promotes an increment of the root and shoot weight in the mencioned cultivars. It does not affect nodulation or Nitrogen Biological Fixation. It behaves as NPR (Nodulation promoting rhizobacteria) in alfalfa and soybean and was isolated as endophyte in wheat , soybean, sunflower and maize.

Pseudomonas aurantiaca is capable of producing a pigment “in vitro”and “in situ” that controls the growth of numerous fungi species such as *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Pythium* spp, *Fusarium* spp and *Alternaria* spp. The pigment is stable in different environmental conditions. One of the compound with antifungic properties is 2, 4-Diacethylphloroglucinol (DAPG). The bacterium produces Indol acetic acid (AIA) and this production is correlated with the presence of the pigment.

Pseudomonas aurantiaca has shown an excellent response in differents crops, being also a very good biocontroller of a wide spectrum of pathogenic fungi. Thus, it becomes in an ideal strain to be formulated as a non-conventional inoculant with the purpose of enhancing plant production through biocontrol and PGPR effect.

It has been formulated as inoculant (BIAGRO SA) and it’s in experimental stage.

P-770 Evaluation of yield crop of *Phaseolus vulgaris* and *Vicia faba* biofertilized with native rhizobia at Tlaxcala, Mexico

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Biofertilization with nitrogen-fixing bacteria in modern agriculture offers great perspectives for recovering soils and to achieve sustainable agroforestry. Considering agronomic cultures, Biological Nitrogen Fixation depending on the rhizobia-legume symbiosis is of great importance, since it increases significantly the levels of soil nitrogen. However, the effectiveness of the symbiosis depends on several steps of mutual recognizing events. Since nitrogen deficiencies appear frequently on perturbed and non-fertilized soils, nodulated legumes own a selective advantage under adverse conditions, growing on restrictive sites to other plants. Sustainable agriculture implies the utilization of several techniques, just as the inoculation of plants with microorganisms reducing the necessity of agrochemical products. Common bean (*Phaseolus vulgaris* L.) is the second most important grain for human feed in Mexico, and its importance is also due to the extent of the cultivated surface. However, other legume grains are useful in nutrition, for example, faba (*Vicia faba*). They also can be grown under different climatic conditions and types of soil if alternative techniques are correctly applied. This work was aimed to evaluate and compare the biofertilizing potential of native specific rhizobia strains on common bean and faba, in two locations in Tlaxcala, Mexico. In addition, the competitive capacity of the strains was evaluated in greenhouse conditions. We followed the efficiency of native strains under different crop conditions. The effects of the inoculation with native rhizobia strains were not evident on the vegetal development, according to statistical analysis. However, the benefits of the inoculation were evident at legume grain production level and on the cultures grown under rotation scheme. Bacterial strains used as biofertilizers were genetically characterized and so their permanence in soil was analyzed.

P-655 Adaptive strategies of rhizosphere processes in different tree species on reclaimed oil shale mining areas

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The restoration of post-industrial landscapes is a challenge. Opencast mining in the Northeast Estonian oil shale field – the largest commercially exploited oil shale deposit in the world – creates substantial areas of wasteland. The relief of the alkaline (pH~8) wasteland is rugged and extremely stony; the N and organic content of oil shale mining spoil is low. Hence afforestation is an optimal tool for the reclamation of these disturbed landscapes. More than 10000 ha of exhausted opencast oil shale mines are afforested mainly by Scots pine (86% of the area), however, deciduous species silver birch (*Betula pendula*) and black alder (*Alnus glutinosa*) are more productive and perspective. Trees must invest assimilates according to an extensive or intensive strategy to optimize their mineral nutrition:

A) Extensive, by increasing the mass, surface area and length of fine roots, leading to the increase of the rhizosphere; B) Intensive, by increasing or maintaining the efficiency of fine roots and rhizosphere processes through morphological adaptations of fine roots and/or the activity of root-associated microorganisms.

The first years are most critical for tree survival in harsh conditions. Hence the chronosequences of one-, four- and 27-yr-old stands were included in the study with the aim of analysing adaptive strategies of rhizosphere processes in different tree species. Biolog Ecoplates were used to determine the community-level physiological profiles (CLPP) in the rhizosphere and bulk soil samples. CLPP were summarized as AWCD (average well color development, OD $48h^{-1}$. The substrate-induced respiration (SIR) and basal respiration (BAS) of bulk soil samples were measured, and the metabolic quotient q(CO2)=BAS/SIR was calculated. Short root morphological studies were carried out using WinRHIZOTM Pro 2003b. The greatest difference between one-, 4- and 27-year-old stands was revealed in the rhizosphere/bulk soil AWCD ratio. It was in 4-yr-old stand 5-10 times higher than in the first year after planting or in a middle-aged stand. SIR increased while $q(CO_2)$ and mean specific short root length (SRl, m g^{-1}) decreased with increasing stand age; soil pH decreased 1-2 units during the first 26 years as well as the acidifying effect of rhizosphere. Hence in improved soil the support of rhizosphere communities is less crucial.

P-730 Biodiversity of indigenous promiscuous soyabean rhizobia in some Zimbabwean soils

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Zimbabwean farmers make use of commercial rhizobium inoculants in the production of soyabeans (Mudimu, 1998). Although the demand for commercial rhizobia inoculant is high farmers cannot access commercial rhizobium inoculant due to distribution bottlenecks and cost. Promiscuous soyabean varieties offer an alternative option by exploiting their ability to nodulate with compatible indigenous rhizobia present in the soil. The exploitation of promiscuous soyabean varieties rests on the assumption that effective compatible rhizobia are present in sufficient numbers in the soil. The study evaluated the occurrence and nodulation potential for indigenous promiscuous soyabean rhizobia in soils collected from three agro-ecological zones. Magoye, a promiscuous soyabean variety nodulated well (100%) in all soils sampled. Seven of the improved varieties nodulated in greater than 75% of the soils. Only 1 variety, SC Edamame nodulated in the least number of soils, about 50 %. Local soils harbour indigenous soyabean rhizobia for all improved soyabean varieties and nodulation differs according to variety. Magoye has the potential to be grown in all soils in the 3 agro-ecological zones in Zimbabwe without the need for inoculation. Improved varieties nodulate in less soils therefore the potential to exploit promiscuity in the different agro-ecological zones and soils is limited.

P-811 Effects of increasing soil salinity and nitrogen levels on grain yield, osmotic adjustments and nutrient uptake in wheat

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The effects of nitrogen rates on grain yield, sodium and potassium, carbohydrate and proline concentration in wheat (Chamran cultivar) was studied in a field experiment in 2004. The treatments were five levels of salinity 1.5, 5, 10, 15 and 20 ds/m as main plots and three nitrogen ammonium nitrate levels 50, 100 and 150 kg $N.ha^{-1}$ as sub plots. Five salinity treatments were used on a clay-loam soil by irrigation water salinized with NaCl and $CaCl_2$ (5:1 rate). Data were collected for shoot and seed samples and analyzed. Results showed by increasing salinity grain yield was decreased, however N application increased grain yield. Grain yield was higher in 150 kg $N.ha^{-1}$ than 50 kg $N.ha^{-1}$ in all levels of salinity. Remoblization of assimilate from stem to seed and harvest index were increased as salinity increased. N had no significant effect on them. As salinity increased, accumulation of carbohydrate and proline in green leaf tissue of flag leaf were increased at flowering stage. With increased N treatments, proline concentration increased but carbohydrate decreased. In this study concentration of potassium decreased in shoot and sodium concentration increased when salinity increased. N had no significant effect on K^+ concentration in seeds. However, concentration of Na^+ in both shoot and seed increased when N application increased. With increasing salinity K:Na ratio was higher in shoot ad low in seeds. It was concluded that the plant growth, grain yield, yield components and osmotic adjustments could depend upon N and levels of salinity in soil. The application of N could possibly be conductive to plant growth in salt affected soils.

P-981 Growth and yield of american cotton (*Gossypium hirsutum* L.) as affected by planting methods and spacings

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The present research entitled "Growth and yield of American cotton (*G.hirsutum* L.) as affected by planting methods and spacings" was conducted at the Students' Research Farm, Department of Agronomy And Agro Meteorology during kharif 2004.The experiment was laid out in split plot design with four replications. The main plot treatments considered of five planting methods viz .sowing on dry beds followed by irrigation, sowing on beds at optimum moisture, sowing on beds made dry and then irrigated for settlement ,furrow planting and flat planting. The sub-plot treatments comprised of three plant to plant spacings i.e. 45, 60, 75 cm. Different planting methods had a non-significant on seedling emergence, plant height, total dry matter accumulation, leaf area index, number of monopodial, sympodial branches, flowers and bolls per plant, setting percentage, lint index, seed index, ginning out turn and total seed cotton yield. Among plant spacings, narrow plant spacing of 45 cm resulted in taller plants with more leaf index than wider plant spacings 60 and 75 cm. However, the wider plant spacing (70 cm) resulted in significantly higher number of sympodial branches per plant, dry matter accumulation (g/plant), number of flowers and total bolls per plant than narrow plant spacings of 60 and 45 cm. Plant spacings had a non-significant on seedling emergence, boll weight, lint index, seed index, ginning out turn, monopodial branches per plant and total seed cotton yield.

P-995 High throughput screening of the effects of changing rhizosphere carbon flow on bacterial antibiotic production

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Rhizosphere carbon flow changes qualitatively over time. It is generally assumed that the ratio of organic (and amino) acids to reducing sugars increases with plant age, resulting in shifting microbial community dynamics. However, how mixed-source C-substrate quantitatively affects functional traits of a rhizobacterial population has not received much attention. We use the model strain *Pseudomonas fluorescens* CHA0 to investigate how the qualitative changes to C-flow affect the production of the antibiotic 2,4-diacteylphloroglucinol (DAPG). This key secondary metabolite is a polyketide with known efficacy against root fungal pathogens. The extraction of DAPG from a growth matrix is time consuming and a rate-limiting step in the development of high throughput systems. We markedly decreased analytical time by using a Porvair Sciences Ltd Microlute® system where the contents of each cell in the growth plate can be transferred to a matching 96 well plate containing C-18 Solid Phase Extraction (SPE) medium that retains DAPG until removed with a suitable solvent for HPLC analysis. We simulated rhizosphere ageing by decreasing the ratio of sugars to acids in 96-well plate format, each well containing an initial inoculum of CHA0. The plates were incubated for seven days and the presence of DAPG in the growth medium quantified by HPLC. The production of DAPG was then related to changing C flow throughout rhizosphere development. We conclude by identifying how the new screening tool can be used to identify strategies for rhizosphere engineering.

P-699 Mesorhizobia adapted to a metallicolous plant : *Anthyllis vulneraria*

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Some human activities can seriously disturb ecosystem dynamics. It is the case of contaminated sites related to mines' exploitation which generate spoils and effluents extremely rich in toxic heavy metals. To limit dispersion of heavy metals that cannot be biodegraded, one method, called phytostabilisation, uses the abilities of metallicolous plants to colonize and stabilize contaminated soils.

The presence on one mine in the area of Saint-Laurent-le-Minier (Gard, France) of a plant-nitrogen fixing bacterium association, *Anthyllis vulneraria-Rhizobium*, able to develop on soil containing high content of zinc, lead and cadmium have a major interest. Indeed, the leguminous plants associated with soil bacteria transform atmospheric dinitrogen into organic nitrogen and could stimulate soil fertility like natural manure. Thus growth of plants is stimulated and could contribute significantly to build a plant cover with root-stabilization of polluted soils.

We compared genetic and phenotypical diversity of the nitrogen fixing bacteria associated with *Anthyllis vulneraria* in mining district from metallicolous and non metallicolous populations. Metallicolous bacterial strains differed from the non metallicolous ones according to taxonomy and abilities for metal tolerances; we observed a low diversity among metallicolous strains. Symbiotic bacteria associated with *A. vulneraria* belong to the *Mesorhizobium* genus and constitute a new bacterial species phylogenetically close to *M. tianshanense*. Phenotypic tests show a high tolerance level to zinc and lead, contrary to strains obtained from non polluted soils.

New isolated *Mesorhizobium* strains show a strong capacity to nodulate metallicolous *A. vulneria* in heavily contaminated soils and are biological materials interesting for phytostabilisation.

P-1019 The influence of mycorrhiza on the growth and yielding of strawberry and the processes taking place in the rhizosphere.

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In 2003-2006 a field experiment was carried out on the effect of mycorrhiza application and different bedding materials (peat substrate, tree bark, saw dust, compost, rye straw) on the growth and yielding of three strawberry cultivars 'Senga Sengana', 'Elsanta' and 'Kent'. All the experimental plots with bedding were fertilized annually in the spring with NPK fertilizers in reduced doses (with respect to the standard NPK fertilization - 70 kg N, 60 kg P and 120 kg K_2O), i.e. 50 kg N, 40 kg P and 50 kg K_2O. Organic bedding materials were applied each year at the rate of 25 l/m^2 along the rows of plants and were mixed with the surrounding soil during spreading. The mycorrhiza substrate was applied to the soil in the root growth zone of each plant (150 mg/plant). The results of the experiment indicate that the application of mycorrhiza and peat substrate or compost bedding to strawberry plants had a beneficial effect on the growth and yielding of 'Senga Sengana' plants, and on the firmness of their fruits. The basic NPK fertilization combined with mycorrhiza application to strawberry plants of that cultivar increased the number of fruits by 21% and the yield size by 16%. The use of peat substrate or mycorrhiza also had a positive effect on root growth, i.e. root length, diameter, surface area, volume, and the number of root tips. The applied organic bedding materials were found to have a beneficial effect on the mineral and organic matter content of the soil.

In a greenhouse experiment, strawberry plants cv. 'Senga Sengana', following mycorrhiza application, had leaves with a more intense green colour, a significantly greater number of leaves, larger leaf biomass, and greater fresh and dry weight, and volume and surface area of roots. The inoculated plants were also characterized by higher acidity of the rhizosphere and a more efficient uptake of mineral ions (P, K, Fe, Zn and Mn) than the control plants (with standard NPK fertilization).

The innovative character of the method consists in the combined application of reduced NPK doses and mycorrhiza substrate to plants and the use of bedding materials (peat substrate and compost) in order to improve the nutritional status, the vegetative growth and yielding of strawberry plants. Using the method in fruit-growing practice will improve the nutritional status of plants, increase their growth and yielding, and as a result it will contribute to the protection of the natural environment.

P-769 Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and wheat and its potential to promote wheat plant growth and crop performance

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Previous studies showed that *Rhizobium leguminosarum* bv. *trifolii* forms a natural, endophytic association with rice that can promote its grain production in a strain-cultivar specific manner. That beneficial association involves the modulation of phytohormone levels in rice, resulting in growth expansion that enables the plant to access and utilize soil nutrients and conduct photosynthesis more efficiently. Here we show that this same rhizobial biovar also forms a natural, endophytic association with wheat, the 2nd most important cereal crop produced in Egypt. Macerates of field-grown, surface-sterilized wheat roots were inoculated on axenic seedlings of a diverse of legume hosts containing fababean, lentil, lupines, fenugreek, pea, berseem clover, soybean and chickpea that are rotated with wheat. Interestingly, true root nodules only developed on berseem clover. Forty-seven clover nodule occupants were isolated into pure cultures and the combination of plasmid profiling plus BOX-PCR genomic fingerprinting grouped them into 3 strain genotypes. The 16S rDNA gene of 8 isolates representing all 3 genotypes was sequenced and all had a high homology to *R. leguminosarum*. All of the isolates nodulated berseem clover in gnotobiotic culture, but only those of the group-2 genotype formed effective N_2-fixing ones. Some isolates in the same group promoted wheat growth in PGPR bioassay. Three consecutive years of inoculation studies in large farmers' fields indicated that some of the group-2 isolates significantly increased wheat grain yield from 16.2% to 39.0%, depending on the variety, strain and geographical location. Inocula consisting of some consortia of wheat-adapted rhizobia performed better than others or than did some single isolates alone. Also, several inoculant formulations significantly increased the agronomic fertilizer-N use efficiency of wheat, indicating that they enhanced the plant's ability to produce grain while reducing its dependence on chemical fertilizer-N to achieve high yields. Considered collectively, these studies indicate that *Rhizobium leguminosarum* bv. *trifolii* is particularly adapted to develop natural endophytic associations with wheat and rice roots in the Nile delta, and some isolates that occupy this ecological niche have high potential for development as biofertilizer inoculants that can improve grain production of these important cereal crops in this geographical region.

P-1127 *Thymelea lythroides* a potential host for *Pisolithus* spp. associated with *Quercus suber* in Morocco

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Quercus suber is of a high interest for local populations in Morocco, both for the value and for the diversity of its products. The anthropogenic pressure exerted over a long time reduces significantly the surface of this species and then restricts its area of its distribution. As a consequence, the physico-chemical and biological soil properties are degraded. Land degradation is usually associated with reduction in the below-ground microbial diversity and / or activity of major biogeochemical cycles. So, natural plant communities become degraded and the processes of desertification are accelerated.

In Moroccan cork Oak woodlands there is many dwarf and tall shrub communities which are characteristic of mediterranean ecosystems. *Thymelea lythroides* is among the frequent shrubs encountered in these areas. Unfortunately, data on this species are not available particularly its mycorrhizal status. So, in our researches, we were interested in this shrub to know if really it can be considered as a vector of mycosymbionts propagation able to facilitate natural regeneration processes of *Quercus suber*.

Nineteen basidiomata of *Pisolithus* spp. were collected under *T. lythroides* in association with *Quercus suber*. The amplification product of the ITS region showed an electrophoretic band of molecular weight ranging between 600 and 700 bp. The analysis of the restriction profiles obtained with Hinf I, Msp I and Taq I enzymes, leads us to differentiate two genetic types within *Pisolithus* spp. According to our data on the ITS sequences of *Pisolithus* spp. associated with Q. *suber*, there are only two species of *Pisolithus* (species 6 and 4, following Martin et al., 2002) that were encountered. The same species of *Pisolithus* were found in association with *T. lythroides* in the Mamora.

We think that the management of the *T. lythroides* shrub layer could represent a benefical factor for the reconstitution of the soil symbiotic microflora and thus to contribute to the regeneration of cork Oak in the Mamora.

P-392 Wetland ecosystem and its rhizospheric environment for generous output and soil-water characterization in Indian subtropics

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Soil, water and biological entity are integral part of global natural resources, determined greatly wetlands, its diversity, habitats of thousands aquatic flora & fauna. Categorically, wetlands are lands transitional between terrestrial and permanent wet body, where soil is frequently waterlogged during rainy months with different water depths. Inland wetlands including coastal ecosystem comprises of 25-30%, intersected with main river system and it's so many tributaries like oxbow lakes (mauns, chaurs, jheels, beels, nayanjali called locally), mostly dominated in the north-eastern Indian sub-continent. These rhizospheric zones are immense valuable, useful for fish-aquatic crop diversity, preserved carboniferous environment of the ecosystem as sources, sinks and transformers of a multitude of chemical, biological and genetic materials and ultimately sustained economic stability to millions of people in the regions, maintaining an ecological balance. Case studies were undertaken on nature and characteristics of wetland ecosystem as per depth and submergence, permanent, semi-permanent and temporary and on production system of popular and enriched aquatic food crops (water chestnut - *Trapa bispinosa* Roxb. and makhana - *Euryale ferox* Salisb.) and fish variables for the benefits and economic stability of farm-families in the regions. Physico-chemical characterization of soil and water in wetlands greatly differs relating to individual characteristics. Indeed, organic matter is high enough but due to anaerobic situation, it partially decomposed. The rhizospheric soil status was further improved allowing certain time for quick decaying of such waste materials during post-wet months and effectively utilized this resourceful humus soil for succeeding arable crops, saves a substantial amount of chemical fertilizer as well as improvement of soil physical condition. Remarkable improvement on fruit and kernel yield, MYE (makhana yield equivalent) and economic output (GMR, NP and B-C ratio) were also exhibited with the system. The soil physico-chemical characteristics are most important for growing such aquatic food crops. All the characters of both soil and water were significantly influenced by depth and duration of submergence as well as rhizospheric characteristics under this investigation. These were most responsive, quite adaptable for both the crops and favourable for effective utilization of these vast waste wetlands safely, who are inextricably linked with system.

P-1164 Effect of ploughing depth on root development

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This work concerns the effect the depth of the work of the soil on the development of the roots of a cereal corn and the consequences on the output of the culture. The tests showed that the piece having undergoes a sub soiling with a 0.50 m depth has a cone index lower than that obtained on the ploughed piece with 0.27 m and that on the latter, cone index increases, after the pseudo ploughings and surface work, up to a value of 13.24 daN/cm^2 on the depth level of 0.13 m the development of the roots was better on the level of the in-depth worked piece. The consequences of the development of the roots was illustrated by an output of 2.787 tons / hectare on the piece worked with a 0.50 meters depth which is higher than that obtained on the piece worked with 0.27 meters and which is only 2.236 tons/hectare, that is to say a difference in 0.551 tons/hectare.

P-1230 Effect of plant growth promoting rhizobacteria on morphological and physiological characteristics and yield of wheat (*Triticum aestivum* L. cv milan)

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Some microorganisms which are present in rhizospher are the cause of a number of physiological and morphological changes in plants. These changes have some positive effects on plant growth, nutrition and health. These microorganisms are called Plant Growth Promoting Rhizobacteria (PGPR). Before now, this term was used for *Pseudomonas fluorescens* bacteria group, but today it has wider meaning and includes bacterias like *Pseudomonas*, *Azotobacter* and *Azosprillum*. This experiment is being carried out in the field with 2 strains of *Pseudomonas putida*, *Pseudomonas fluorescens* and *Azotobacter*. Treatments include :

1)NPK without inoculation(control)
2)NPK + *P. putida*1
3)NPK + *P. putida*2
4)NPK + *P. fluorescens*
5)NPK + *Azotobacter*
6)NPK + *Azotobacter* + *P. putida*1
7)NPK + *Azotobacter* + *P. putida*2
8)NPK + *Azotobacter* + *P. fluorescens*

Results showed that inoculated wheat seed with *Pseudomonas* and *Azotobacter* increased yield and improved physiological properties (N , Fe , Chl, Car , Pro , GB content and nitrate reductase activity) and morphological properties such as tillering and biomass.

P-1296 Effect of different planting times and isoflavon genisteine concentrations in root zone on nodulation and nitrogen fixation of three *Medicago* species

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In order to study the effect of planting times and genistein on nitrogen fixation, nodulation and other traits related to three annual medics, a field experiment was conducted in 2004-2005. In this experiment 3 annual medics, 3 levels of planting times (February 20, March 1 and 10) and 2 levels of genisteine (0 and 20 µM) were considered for use in the root zone. Field experiment was conducted in split-split plot design in base of completely randomized block with four replications. In field experiment, varieties were significantly different for nitrogen fixation, nodulation and other traits. *M polymorpha* was better than other varieties for nitrogen fixation and nodulation, root dry matter production, and nitrogen percent. *M polymorpha* had a greater ability for nitrogen fixation than other varieties. Second and third planting times yielded greater nitrogen fixation and forage production than the first planting time. The application of genistein at 20 µM compared with the control (0 µM) was more effective on nodulation and nitrogen fixation under field conditions. This experiment showed that genistein was more effective at environmental low temperatures on nodulation and nitrogen fixation of annual medics.

P-1295 Effect of bentonite and chickpea on the content in nitrogen of durum wheat cultivated in sandy soil

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The Mostaganem land sandy soils have a low clay content. Addition of bentonite in these soils and durum wheat-chickpea culture association, constitute the two ecophysiological strategies to rehabilitate this agricultural region. The changes in available nitrogen contents (NH_4^+ and NO_3^- nitrogen) in soils with our without addition of 10% bentonite have been analysed during the development of the two species. Results show that the NH_4^+ and NO_3^- nitrogen in the soil are high in association culture at all plant development stage and with bentonite application. The amount of nitrogen in the soil is higher in the soil with 10% bentonite addition at tillering stage.

P-1197 The effect of a new slow release compost, fulvic acid and micro-organisms on soil fertility, yield and quality of potatoes grown in sandy soil

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The commercial production of potatoes in South Africa is mostly done on sandy soils that contain less than 5% clay with almost no organic matter content (% C < 0.1%). This results in low binding (low CEC) and retention of plant nutrients as well as inefficient nutrient exchange and utilization by plants. Therefore high application of chemical nutrients is standard practice in potato production to achieve acceptable yields. Furthermore, due to the low organic matter content and high free salt concentration in the soil solution, microbial activity is totally absent resulting in sub optimal root development. In practice the high nutrient and water application requirement per kg harvested are becoming prohibitively expensive.

It was hypothesized that by restoring balance and synergy in the soil through firstly a new long lasting slow release compost not containing any manure or straw using fresh wood fiber and selected micro-organisms, secondly a unique high concentration fulvic acid developed using a renewable plant resource (phytofulvate) and lastly to complete the soil applied treatment with a combination of specific rhizosphere micro-organisms, this equilibrium farming and synergy concept would restore soil balances, improve nutrient and water uptake, increase yield and quality including mineral nutritional value. An extra treatment was added where micronutrients and growth stimulants were foliarly applied.

In this statistical trial done at the SAND Experimental Farm a highly significant best treatment (soil plus foliar) increase in yield of 24.7 ton/ha (43%) was achieved. Compost alone yielded 12% (3.56 ton/ha) higher than the control while the addition of fulvic acid and microbes resulted in 25% (14.19 ton/ha) increase in yield. The soil treatment had a direct effect on soil chemical changes (higher CEC (15%) and %C (38%), lower resistance (-38%)). Mineral nutrient retention in the soil eight months after treatment was between 7% (Zn) and 46% (Mn) higher than in the non-treated soil. Content of certain minerals also increased in harvested potatoes resulting in improved nutritional value to the consumer. The synergism and symbiosis between micro-organisms and potatoes grown in a balanced soil environment are evident in the results.