Effects of climate change on Agriculture

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Looking into the future of agriculture raises three challenging questions: How can agriculture deal with an uncertain future? How do local vulnerabilities and global disparities respond to this uncertain future? How should we prioritise adaptation to overcome the resulting future risks? This lecture analyses the broad question of how climate change science may provide some insights into these issues. The data provided for the analysis are the product of our new research on global impacts of climate change in agriculture. The questions are analysed across world regions to provide some thoughts on policy development.

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Impact of agriculture on climate change

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In order to determine the role of agriculture on climate change, it is important to consider the impact of land use and land management on climate. For example, the type of vegetation, method of tillage, amount of land cover, are all factors that influence climate by changing transpiration, precipitation, air temperature and net radiation. These changes can alter the global climate if the energy budget at the Earth's surface is significantly changed. There are a whole series of forcing agents that have the potential to affect the energy budget and the Earth's temperature. Some forcing agents such as greenhouse gases (GHGs) cause warming (the biogeochemical effects) while others that cause an increase in the reflectivity of the surface (albedo) mainly cause cooling (the biogeophysical effects) (Desjardins et al., 2007).

Globally, agriculture presently accounts for 13% of the radiative forcing related to GHGs. In the past, intensive agriculture (e.g. deforestation, cultivating grasslands and planting rice) have contributed significantly to the increase in atmospheric carbon dioxide (CO_2) and methane (CH_4). The global potential to mitigate the greenhouse effect is substantial (Smith et al., 2008). In many countries, agricultural GHG emissions are now mainly in the form of CH_4 and nitrous oxide (N_2O). Agricultural sources such as animal husbandry, manure management and agricultural soils account for about 52% of global CH_4 and 84% of global N_2O emissions. Microbes play an important role that results in GHG emissions. By making it possible for cattle to utilize forages, as an energy source for maintenance, growth and milk production, they produce CH_4. Other microbes fix nitrogen from the air. It is through this process that a hundred million metric tons of usable N compounds are taken from the atmosphere for use by living beings each year.

Agricultural activities can also influence climate through land use change, which can modify the albedo of the Earth's surface. Any combination of factors that result in an increased albedo mean that less solar energy is absorbed by the Earth's surface. Compared to the globally averaged albedo of about 0.3 for the Earth's surface, land covers with higher albedo (such as snow and ice, $\alpha = 0.90$) tend to lower the air temperature, while land covers with lower albedo (such as oceans, grasslands and forests, $\alpha = 0.05$ to 0.20) tend to increase air temperature. It is estimated that a 0.005 reduction in global albedo would modify the shortwave radiation forcing by about 1.7 W m^{-2} and cause an increase in the global air temperature by about 0.9 ºC.

We will present several examples of the diversity of human climate forcing. Many programs have been initiated to mitigate GHG emissions and so far considerable progress has been reported in reducing the GHG emission intensities from agricultural sources, but because of increasing food demand and increasing energy requirements, the total GHG emissions from agriculture keep increasing (Desjardins, 2010). So far, mitigation measures have been biased towards minimizing the biogeochemical effects but there is growing awareness that the biogeophysical effects may also be important. Better information on the various types of forcing is particularly important to help decisions that the farming community will have to do in order to minimize the impact of agriculture on climate change.

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The N and C biogeochemical cycles and climate change

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Several drivers, or forcing agents, can affect the Earth's biological, chemical and physical processes at a planetary scale. Humans are now the prime driver of global change, which involves climate change, reduced water quality and availability, biodiversity loss, and degraded ecosystem.
The nitrogen (N) cycle and the carbon cycle are closely linked and both are changing rapidly. In the last six decades, human production of Nr has outstripped production from all natural terrestrial systems. At the same time, rapid increases of emissions of man-made greenhouse gases have been observed which are linked to climate change. Nitrogen-cycle changes and greenhouse gas emissions have the same drivers: population growth, changes in diet, increasing demand for energy, for food, livestock feed and fiber, which drive land-use change.
Nitrogen is important in many ways: it plays a major role in the production food and energy. Human activity has doubled the level of Nr in circulation, largely as a result of fertilizer application, fossil fuel burning and widespread cultivation of legumes, rice and certain other N-fixing crops, and increased livestock feeding and manure production. This massive alteration of the nitrogen cycle affects climate, food security, energy security, human health and ecosystem services.
The long-term consequences of such large changes to the nitrogen cycle have largely been failed to include in global environmental assessments and climate policy. This presentation focuses on climate interactions, particularly with the carbon cycle, but includes costs and benefits of N-cycle changes to other parts of the Earth system. The latest research suggests improved international nitrogen management will be part of the solution to tackling climate change. The question is, how does artificial Nr production exacerbate the climate-change problem? And how does Nr alter mitigation and adaptation options to address climate change?

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Plant Growth-Promoting Microorganisms

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As the world's population continues to increase, to feed all of the world's people, it is imperative that agricultural productivity is sustainably increased. One of the strategies that will be important in this endeavor is the increased use of plant growth-promoting bacteria. In recent years scientists have developed a more profound understanding of the basic mechanisms that are employed by these bacteria to facilitate plant growth. One of those mechanisms, the ability of plant growth-promoting bacteria that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower plant ethylene levels, often a result of environmental stresses, is a key component in the efficacious functioning of these bacteria. In addition to some of the more well studied mechanisms of bacterial plant growth promotion such as nitrogen fixation and siderophore sequestration of iron, the optimal functioning of these bacteria depends on the synergistic interaction between ACC deaminase and indole-3-acetic acid. Using these mechanisms, bacteria not only directly promote plant growth, they also protect plants against flooding, drought, salt, flower wilting, metals, organic contaminants, and both bacterial and fungal pathogens. While a considerable amount of both basic and applied work remains to be done before ACC deaminase-producing plant growth-promoting bacteria become a mainstay of plant agriculture, all of the evidence indicates that with the expected shift from chemicals to soil bacteria, we are on the verge of a major paradigm shift in plant agriculture.

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Biological Nitrogen Fixation by the Legume-Rhizobia Symbiosis

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Nitrogen is a major limiting factor in agricultural soils despite its abundance in the atmosphere as inert dinitrogen (N_2), and the availability of nitrogen reactive forms in soils has conditioned human history. The development of mineral nitrogen fertilization (produced through the Haber-Bosch process) has been crucial for the Green Revolution and has diminished famine in many regions of the world. However, this has been at the expense of important environmental costs, illustrated by the growing accumulation of nitrates in soils and water. Biological Nitrogen Fixation (BNF) constitutes an alternative, natural process for N_2 reduction to ammonia. This biological ability is restricted to some prokaryotes that are able to break the strong triple bond of the N_2 molecule, in a process catalyzed by the enzyme nitrogenase. The process requires high doses of energy; locally anoxic conditions are also needed, since nitrogenase is rapidly inactivated by oxygen. Several genera of *Bacteria* and *Archaea* have been described as diazotrophs able to use molecular nitrogen as a nutrient, either as free-living microorganisms or in mutualistic symbioses with plants. These include: i) plant associations with N_2-fixing bacteria living inside the plant cell (endosymbioses); ii) the so-called associative symbioses; and iii) the cyanobacterial associations with *Azolla* or with non-legume plants. Within the endosymbioses, the *Rhizobium*-legume association is by far the most important and probably the most efficient nitrogen-fixing system, considering both the profitability and the greater environmental sustainability that legume nitrogen fixation provides.

The establishment of an efficient *Rhizobium*-legume symbiosis is a complex process that ends with the formation of the nitrogen fixing root nodule, a plant organ resulting from induction of specific developmental programs in both symbionts. Several signals are exchanged between plant and bacteria, in a signal transduction process reminiscent of ancient plant-mycorrhizal fungi communication programs. This signaling induces the formation of an infection thread through which the bacteria reach the nodular cortex where they are engulfed by plant cells. There, a plant-derived membrane surrounds the bacteria, resulting in the formation of the minimal symbiotic unit, the symbiosome. Multiple morphological and metabolic changes occur, leading to an arrest in bacterial growth and to the formation of bacteroids. Inside the nodule, bacteroids find the ideal, ultra-microoxic environment to fix nitrogen, with abundant carbon substrates that provide energy and electrons for the reduction of N_2.

All agriculturally important legumes can fix nitrogen in symbiosis with their rhizobial counterparts. Several approaches can be followed to improve BNF by legumes, such as wider legume adoption practices, plant breeding and selection, and the use of selected rhizobial strains as inoculants. Successful adoption of legumes is reported from all continents, where they deliver profitability and often provide multi-purpose benefits to farmers: establishment of new dryland cereal-legume cropping and intercropping systems; reduction of energy-intensive nitrogen fertilizer use; improvement of water use efficiency; and integration of crop and livestock production in mixed systems, among other. In a more general context, both a full understanding of BNF in legume crops and the extension of its benefits to other agriculturally important (e.g., graminaceous) species are required.

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Legume Inoculants

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Legume inoculants based on different *Rhizobium*/*Bradyrhizobium* strains, in general rhizobia, are highly biotechnological products, worldwide used to increase yields of grain and forage legumes in a frame of sustainable agriculture. Formulation and use of these inoculants date back 19th century.

Advantages and constrains of the practical application of such biological fertilizers will be discuss and examples of their usefulness and limitations presented. The general biogeochemical N and C cycles and the BNF process, as background of this agriculture practice, will be presented in previous presentations.

Other microbial inoculants based on labeled Plant Growth Promoting Rhizobacteria (PGPR) will be introduced. The formulation and use of this plethora of microorganisms is an emerging task in areas such as crop protection, plant nutrition, bioremediation and cropping under environmental stress conditions, then examples of the microbial species and the target crop and challenges will be also addressed.

Especial emphasis will be paid to formulation, quality control and modes of application of legume inoculants throughout the presentation.

The unique experience of legume inoculants production in Spain will be introduced as a small scale system to provide local market with high quality legume inoculants.

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Breeding legumes for enhanced nitrogen fixation

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The economic value of the N fixed by rhizobia in symbiosis with agricultural legumes is substantial. Herridge et al. (2008) estimated that 186 Mha pulse and oilseed legumes and 110 Mha pasture and fodder legumes fix a total of 33–46 Tg N annually, with a nominal value of US$50–70 billion. Although legume N_2 fixation is, to a large extent, determined by the availability of water and the agronomic management of the crop or pasture, the concept of adding more value to legumes through selection and breeding for enhanced N_2 fixation was advanced more than 60 years ago. Various programs have sought to genetically improve a range of species, from pasture legumes such as red clover (*Trifolium pratense*) to the crop legumes soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*). In some, the selection trait was plant yield, whilst in others it was high plant reliance on N_2 fixation (%Ndfa).

A third strategy was to optimise legume nodulation through specific nodulation traits. The cultivation of legumes in many agricultural soils during the past 5,000 years, in concert with purposeful and accidental inoculation of the legume seed and soil with compatible rhizobia, has meant that few soils are now totally devoid of rhizobia that can nodulate the legumes grown in those soils. That is not problematic if the soil rhizobia are effective with the sown legume and rates of N_2 fixation are near optimal. Problems do occur, however, when the soil rhizobia are infective but sub-optimal in fixing N_2 with the legume host. In such situations, inoculation of the sown seed or soil may enhance N_2 fixation when the soil populations are low, but may have no, or at best temporary, effects when soil populations of rhizobia are moderate to high.

Various research programs addressed this problem of the legume and its relationship with the naturalised soil rhizobia. Notable are those that aimed for selection and breeding of either selective (Lohrke et al. 1996) or promiscuous nodulation of soybean (Mpepereki et al. 2000). A current program in Australia aims to define the extent to which advanced breeding lines and released cultivars of pasture, e.g. subterranean clover (*Trifolium subterraneum*), and crop legumes, e.g. pea (*Pisum sativum*), form effective symbioses with diverse populations of naturalised soil rhizobia and with the current inoculant strains (Drew and Ballard 2010). The long-term objective is to use high-performing lines/cultivars for breeding.

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Inoculants for cereals

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Inoculation of plants with plant growth-promoting bacteria (PGPB) and plant symbionts to enhance performance of plants is centuries old. Inoculation of cereals, mostly with PGPB is four decades old and these inoculants are not different in general terms from those of other plant species. Yet, the majority of field inoculations done today are on cereals.

In general, shortly after suspensions of bacteria, the most primitive inoculant, are inoculated into the soil without a proper carrier, the bacterial population declines rapidly for most species of PGPB. This phenomenon, combined with production of bacterial biomass, the need to sustain activity in the rhizosphere, and the physiological state of the bacteria at application time, can prevent the buildup of a sufficiently large PGPB population in the rhizosphere. These unprotected, inoculated bacteria must compete with the often better-adapted native microflora and withstand predation by soil microfauna. Consequently, a major role in the formulation of inoculants is to provide a more suitable microenvironment, combined with physical protection for prolonged periods to prevent a rapid decline of bacteria introduced into the soil. Inoculants for field-scale use have to be designed to provide a dependable source of bacteria that survives in the soil and become available to the plant, when needed.

The first goal when considering inoculation of cereals with PGPB is to find the best strain of bacteria or a microbial consortium for the intended effect on the target crop. The next step is to design a specific inoculant formulation for specific target plants and a method of practical application, considering the limitations of the growers (Bashan et al. 2014). Currently, many inoculants for cereals are in the marketplace, some that substantially improve cereal yield.

In the last decade, several reviews summarized the field of cereal inoculation. Most have concentrated on specific bacterial genera, such as *Azospirillum* (Bashan et al., 2004; Bashan and de-Bashan, 2010), availability of various PGPBs and their modes of action (Andrews et al., 2003; Lucy et al., 2004; Vessey, 2003), reduction in the use of fertilizers by supplementation with inoculants (Adesemoye and Kloepper, 2009), and potential marketing (Berg, 2009).

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Microbial endophytes, the inside aid

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Endophytic bacteria reside within living tissue of plants without substantively harming them. They are of high interest for agro-biotechnological applications, *e.g.* for improvement of plant growth and health, phytoremediation, or even as biofertiliser. The supply of atmospheric N_2 to cereal crops is an agronomically and ecologically important potential of N_2-fixing grass endophytes, which has been in part been demonstrated *e.g* for *Gluconacetobacter diazotrophicus* and sugar cane, or *Azoarcus* sp. strain BH72 and Kallar grass. The lifestyle of these endophytes is remarkable, as they colonize the intercellular spaces and vascular tissues of plants in high numbers; however the molecular mechanisms by which they interact with the plant are not yet well understood (1).

A metagenomic approach to analyze an endophytic bacterial community resident inside roots of rice, one of the most important staple foods, allowed to predict traits and metabolic processes important for the endophytic lifestyle, suggesting that the endorhizosphere is an exclusive microhabitat requiring numerous adaptations. Prominent features included flagella, plant-polymer-degrading enzymes, protein secretion systems, iron acquisition and storage, quorum sensing, and detoxification of reactive oxygen species (2). *Azoarcus* sp. strain BH72, a mutualistic endophyte of rice and other grasses, is a well-studied diazotrophic model endophyte (3). For symbiotic interactions with rhizobia and arbuscular mykorrhiza (AM), common plant signaling cascades are now well characterized. Our results suggest that the cascades are not operating during endophytic interactions. Nevertheless, transcriptomic analysis demonstrated that both partners show extensive metabolic adaptations during endophytic interaction. Transcriptomic analysis demonstrated that partners show extensive adaptations during interaction. Upon exposure to exudates, an overall expression of 4.4% of the 3992 protein coding genes of *Azoarcus* sp. strain BH72 was altered. Genes with modulated expression included a large fraction encoding proteins with putative or unknown functions. Mutational analysis of several differentially regulated genes like those encoding a minor pilin PilX, signal transduction proteins containing GGDEF domains and a serine-threonine kinase as a putative component of the type 6 secretion system (T6SS), revealed their role in host colonization. The T6SS was analyzed in more detail. *Azoarcus* sp. harbors two T6SS gene clusters. Mutational analysis showed that the T6SS affects colonization of rice roots (4). Additionally, strain BH72 appears to show quorum-sensing like, density-dependent gene regulation using a small hydrophilic inducer (5). Several of the affected genes were also upregulated in association with rice roots. Our data suggest that strain BH72 may be primed for the endophytic lifestyle by exudates.

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Mycorrhizas, the extended roots

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Soil degradation and nutrient depletion in large areas around the world as a consequence of the continuous indiscriminate land exploitation, abuse in the use of agrochemicals and production of waste pollutants is nowadays one of the most crucial agro-ecological and economical problem to which humanity is confronted (Jeffries et al., 2003). In recent years there has been an increasing awareness/concern by general public and governments about the necessity of sharply reduce such degradation, while maintaining economic activity to socially-acceptable levels. As a consequence of this, new and strong legal initiatives have been presented in order to stop/reduce such an irrational use and/or production of chemicals and waste. At the same time, alternative biotechnological tools are strongly demanded in order to support restoration/rehabilitation of degraded land zones and to manage agricultural fields in a more sustainable manner.

Mycorrhizal symbiosis is a well known, widespread beneficial interaction between a restricted group of soil fungi (the glomalean fungi) and roots of most of the economically interesting plants. Many scientific reports have confirmed that the correct functioning of this symbiosis confers a better nutritional and health status to plants, increasing crop yield and quality of fruits. Much economic resources have been invested by Governments and Research Institutions in order to study possible applications of these microorganisms as biotools. However, extensive application of mycorrhizas in agriculture and environment restoration has been up to now hindered by the lack of adequate inoculum. This should be mainly attributed to the fact that AMF are obligate symbionts, being unable to proliferate in the absence of an appropriate host root and thus requiring special culture systems which were not fully available until late 1990's (Cano et al., 2008). Indeed, the development of arbuscular mycorrhiza monoxenic cultures (Declerck et al., 2005), allowing AMF to grow under *in vitro* conditions on agar-based media, has enlarge our view on arbuscular mycorrhizal (AM) fungal genetics, physiology and developmental features. These culture systems have also been the starting point for the industrial production of ultrapure, tailor-made arbuscular mycorrhizal inoculants (Bago & Cano, 2006; González et al., 2007).

After more than 20 years of scientific research at the Spanish Reasearch Council a patent was presented in 2005 for the design and producion of new in vitro-raised mycorrrhizal inoculants containing autochthonous fungi. These innovative products, now commercialized under the generic name MYCOGEL® have been tested under agronomic conditions, rendering important benefits in crops. This is a clear example of benefits obtained by applying biotechnological tools involving microorganisms to agriculture and environment.

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Microbial pesticides

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Actual crop losses due to pests, diseases and weeds, despite crop protection measures, represent 28-40% of the global agricultural productivity. Conventional pest and disease control have been routinely achieved by using synthetic chemical pesticides, but current approaches are focused to the rational use of pesticides and the reduction of the number of authorized compounds for plant protection. Furthermore, plant pests and pathogens often develop resistance to insecticides, fungicides or bactericides that may compromise its control in several crops. Thus, there is a strong need to develop novel pesticide products which fulfil the strict regulations about toxicity, environmental impact and biodegradability compiled in national regulations mainly in USA and Europe.

Microbial pesticides are currently an alternative or complement to conventional pesticides for plant pest control. They are prepared from microbial strains of several plant associated microorganisms that exert antagonistic or competitive abilities against plant pathogens or cause disease in pests. In fact metagenomic studies have revealed a high diversity of microbiota associated to plant environments, ranging from 100 to 1000 OTUs, of which we are able to grow only a minor part. Many microbial strains that have been developed as biopesticides have mechanisms of action based on antibiosis, competitive exclusion, direct interaction with the pathogen/pest, signal interference or host-mediated resistance.

The use of microbial pesticides is strictly regulated in the EU and it is required a risk evaluation procedure performed by the European Food Security Agency (EFSA). Currently, up to 40 microbial strains have been authorized in the EU as bactericides, fungicides or insecticides, that represent only the 6% of plant protection active ingredients approved (http://ec.europa.eu/food/plant/pesticides). The low presence of these products in the market, in spite of the bureaucratic difficulties for registration of microbial pesticides, is due to (1) the low/moderate efficacy of pest and disease control compared to the conventional products, (2) the frequent inconsistency of results due to its living nature and complex interactions, (3) the concerns about biosecurity in certain microbial species, and (4) the limited shelf-life and stability of the formulated products compared to the conventional synthetic pesticides.

However, research oriented to solve these problems is improving formulation of microbial pesticides, increasing their shelf-life and ecological fitness in the field. Also, because the active ingredients of microbial pesticides are specific strains of a given species, there are no conventional microbiological methods to distinguish them from the authoctonous parents, needed for its specific analysis in the field or in plant products that arrive to the consumers. Genomic studies of several strains have revealed strain specific molecular markers to develop quantitative analysis methods like real time PCR, NASBA or LAMP, providing tools for its traceability, dispersion, and environmental impact studies. Also, the advances in genomics, transcriptomics and proteomics, are also helping to improve efficacy by discovering new mechanisms of action and strains, and to better evaluate biosecurity risks.

Microbial pesticides offer great expectations as tools within the integrated pest protection (IPM) programs, and will contribute in the future to face with the new pests and diseases that will continuously appear due to market globalization and climate change.

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The nitrogenase system: facts and challenges

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Two identified barriers have traditionally impaired the approach of engineering nitrogen fixation directly into crop plants: the known sensitivity of nitrogenase to O_2 (the byproduct of plant photosynthesis) and the apparent genetic/biochemical complexity of nitrogenase biosynthesis.[1] This talk reviews how recent advances in our understanding of nitrogenase biosynthesis offer a new perspective over this ambitious agronomical objective.

The majority of biological nitrogen fixation ($N_2 + 8e^- + 8H^+ + 16$ ATP □ $2NH_3 + H_2 + 16$ ADP + 16P) is catalyzed by the molybdenum nitrogenase, an enzyme composed of two oxygen-sensitive metalloproteins termed NifH and NifDK. NifH is a homodimer that carries a [Fe_4-S_4] cluster at the subunit interface whereas NifDK is an $\alpha_2\beta_2$ heterotetramer that carries one P-cluster [Fe_8-S_7] and one FeMo-co [Mo-Fe_7-S_9-C-homocitrate] in each αβ half of the protein.[2] NifH acts as specific electron donor to the NifDK component, which reduces substrates at the FeMo-co sites.[3]

A number of nitrogen fixation (*nif*) gene products are required to mature apo-NifH and apo-NifDK polypeptides into their catalytically active forms.[4] In the model diazotrophic bacterium *Azotobacter vinelandii*, maturation of apo-NifH into NifH requires NifM, NifU and NifS. NifS is a cysteine desulfurase that provides S for cluster assembly while NifU serves as molecular scaffold to hold the nascent clusters. It has been shown that NifU transfers newly synthesized [Fe_4-S_4] clusters to apo-NifH to generate the holo-NifH. NifU and NifS are also involved in the initial steps of P-cluster and FeMo-co syntheses for the NifDK component (see below). NifM is a peptidyl-prolyl cis-trans isomerase proposed to change the conformation of apo-NifH to allow insertion of its [Fe_4-S_4] cluster.
Maturation of apo-NifDK into NifDK is a stepwise process initiated by the formation of the P-cluster and completed by the incorporation of FeMo-co into apo-NifDK. NifU and NifS provide [Fe_4-S_4] clusters that will serve as P-cluster precursors. P-cluster assembly occurs directly onto the apo-NifDK protein and is directed by NifH. FeMo-co is sequentially assembled at the scaffold proteins NifU, NifB and NifEN prior to its incorporation into apo-NifDK.[4] Substrates for FeMo-co synthesis, i.e. S, Mo and homocitrate, are provided by NifS, NifQ and NifV, respectively. Two proteins, NifX and NafY, protect and carry FeMo-co biosynthetic intermediates during synthesis. Surprisingly, only three proteins, NifB, NifEN and NifH were shown to be essential to FeMo-co synthesis.[5] This finding led to a substantial simplification of this pathway and -together with the fact that we now posses tools to assay each step of nitrogenase biosynthesis- has opened the possibility to carry out a fully-controlled engineering strategy in crop plants. One challenge is merging the late steps of FeMo-co synthesis into the pathway of [Fe_4-S_4] cluster synthesis present in all eukaryots. Another challenge is to find a correct subcellular environment that will allow nitrogenase to function. Two places to introduce nitrogenase into the plant will be discussed: chloroplasts and mitochondria.

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Prospects for engineering nitrogen-fixing cereals

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After water, available nitrogen is the most limiting nutrient in the biosphere for plant growth. Although the demand for fixed nitrogen can be met by chemical fertilisers, anthropogenic disturbances to the nitrogen cycle have resulted in major environmental and economic impacts. At present less than half of the nitrogen used by farmers is assimilated by crops. Excess nitrogen leaks into ecosystems, leading to significant effects on soil and water quality, biodiversity, and atmospheric pollution. Biological nitrogen fixation, catalysed in bacteria and archaea by the enzyme nitrogenase, provides a more sustainable alternative to the use of synthetic fertilisers, but efficient plant microbe symbioses are predominately restricted to legume crops.

Endowing cereal crops with the capacity to fix nitrogen is a long-term challenging goal requiring complex synthetic biology to manipulate the many genes involved. In addition it will require provision of an appropriate environment within the plant to provide sufficient carbon to fuel nitrogen fixation, without subjecting the nitrogenase enzyme to oxygen damage (1). Currently, we can consider three different approaches to this problem: (a) engineer synthetic associations between diazotrophic endophytic bacteria and cereals (b) engineer the legume root nodule symbiosis in cereals, and (c) introduce the complete biosynthetic pathway required for nitrogenase activity into cereals.

In this talk, I will discuss approaches towards engineering expression of nitrogenase activity in cereal crops. Stable expression of Nif proteins in plant chloroplasts or mitochondria could be achieved by targeting nuclear-encoded polypeptides to these organelles. Alternatively, chloroplast transformation could be considered, which would have potential for the expression of polycistronic *nif* operons. Advances in our understanding of nitrogenase mechanism and metallocluster biosynthesis (2, 3) have made it possible to assess the activity of each Nif protein expressed in plants and determine whether there are any roadblocks towards the assembly and function of nitrogenase in plant organelles.

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ENSA: Engineering the Nitrogen Symbiosis for Africa

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The John Innes Centre is leading a project sponsored by the Bill & Melinda Gates Foundation to test the feasibility of developing cereal crops capable of fixing nitrogen, as an environmentally-sustainable approach for farmers in sub-Saharan Africa to increase maize yields. The ENSA project is a collaboration between the John Innes Centre, Aarhus University, INRA-CNRS, University of Wisconsin-Madison and the Danforth Centre. It will initiate the first steps towards the transfer of biological nitrogen fixation to cereals, through engineering nodulation signalling in maize and Setaria viridis, an emerging model system for the engineering of Panicoid grasses.

Small-scale farmers in sub-Saharan Africa neither have the resources to buy inorganic fertilisers, nor the infrastructure for their production and supply and yields remain very low. A symbiosis providing even low level of symbiotically fixed nitrogen could have a significant impact of crop yields in the region. Engineering a maximally efficient nitrogen-fixation system would requires four major step: engineering the signalling pathway to allow recognition of rhizobial bacteria; engineering the production of nodules; facilitating rhizobial infection and producing an environment conducive to nitrogen fixation. The ENSA project is building on the first two steps in this process.

The ENSA project uses the tools of synthetic biology combined with the gain-of-function mutations in symbiosis signalling genes which allow the isolated study of component parts of the Nod factor signalling pathway: Nod factor activation of calcium oscillations, calcium activation of gene expression and cytokinin induction of cell division. The work builds on the knowledge that cereals already possess the symbiosis signalling pathway and readily establish the mycorrhizal symbiosis. In addition, mycorrhizal fungi produce lipochitooligosaccharide (LCO) signals similar to Nod factors indicating that many of the mechanisms recruited for nodulation signalling in legumes are already present in cereals. This work will allow us to assess what the minimal requirements are for Nod factor induction of the symbiosis signalling pathway and whether the symbiosis signalling pathway engineering is sufficient for nodule organogenesis.

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Closing notes: The era of microbial biotechnology in agriculture

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An intensive agricultural production is essentially necessary to satisfy food requirements for the growing world population, thus modern agriculture is nowadays being implemented at a global scale. However, intensive agriculture is associated to the mass consumption of energy, fossil fuel, rock phosphate reserves, water, forested area, and topsoil, and with the emission of greenhouse gases generating climate changes. Therefore, different research approaches are being undertaken addressed to meet environmental and economical sustainability issues, trying to save at most as possible usage of non-renewable natural resources, but without compromising on yields. A feasible and effective approach toward developing sustainable practices in modern agriculture is that based on exploiting the interactions between soil microbial communities and crops, as will be discussed in this Symposium.

Actually, basic, strategic and applied research has demonstrated that some microbial activities can successfully be manipulated, as a low-input biotechnology, to help sustainable environmentally-friendly agro-technological practices thereby benefiting plant fitness and soil quality. Nowadays, and with a clear projection to the nearest future, it is recognized the beneficial impact of microbial inoculants in sustainable agriculture. For a proper application is fundamental to explore first the genetic and functional diversity of microbial communities in the rhizosphere, and to go deeper on the molecular basis of plant–microbe interactions. Selected rhizosphere-adapted microorganisms will continue being used in view of the potentiality of their attributes concerning: (i) promotion of plant growth and nutrition (legumes, cereals, plantation crops…); (ii) biocontrol of diseases, pests and weeds; (iii) plant protection against salinity, drought and extreme temperatures; (v) bioremediation; and (iv) improvement of soil structure. Both rhizospheric bacteria and fungi, either saprophytic or endophytic symbionts (with special reference to N_2-fixing rhizobia and mycorrhizal fungi) will be protagonists of applied microbial biotechnology in agriculture. Particular emphasis will be paid in the future to formulation, quality control and modes of application of microbial inoculants. Engineering novel plant-microbe symbioses, particularly N-fixing cereals, will be critical strategies in the nearest future. Other aspects associated with microbial biotechnology, fundamental in the current scenario of climatic global changes, such as ecosystem restoration, induced resistance to environmental stresses, enhancing plant resilience, recovery of endangered flora, adaptive strategies for biodiversity conservation, etc., must also be considered. Many achievements have been reached with the application of microbial biotechnology in agriculture and in ecosystem restoration, but many challenges as well as opportunities need to be explored.

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