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Front cover: The photograph taken by Mr. Amado Vergara exhibit Hereford steers strip grazing a mix pasture of birdsfoot trefoil (Lotus corniculatus L.) and white clover (Trifolium repens L.) in Uruguay. The **Special issue** is based on abstracts arising from the International Workshop Integrating Genomics into Plant Breeding held on 25 and 26 October 2007 in Porto Alegre, Brazil, and financed by the Sixth Framework Programme (European Union).

Lotus Newsletter (2007) Volume 37 (3), i-v.

Background

Livestock production depends on grazing systems. In South America the area for grazing has been reduced by the expansion of intensive crops systems, pushing livestock towards less fertile and degraded areas. Under this reality, there is an increasing need for selection of productive forage species adapted to stressed environments.

The value of legume forage in low fertility soils is based on its capacity to fix atmospheric nitrogen. Compared to other legumes, *Lotus spp* have:

- good growth in low phosphorous soils;
- better adaptability to acid soils;
- potential for adaptability to other abiotic stresses;
- optimal tannin content.

Objectives of the Workshop

This Workshop is part of the activities of the LOTASSA project (www.lotassa.org), which is in its second year of execution.

The objectives are:

- to share and scrutinize preliminary results and achievements among the scientific community;
- to complement the LOTASSA project with the achievements of other scientific groups working in related areas;
- to establish contacts and to know the vision from the seed and inoculants industry as well as from the private sector involved in forage and animal production in the region.

THE WORKSHOP WILL BE IN ENGLISH AND NO INTERPRETER FACILITIES WILL BE AVAILABLE.

ENTRANCE FREE BUT PREVIOUS REGISTRATION IS REQUIRED.

FOR ADDITIONAL INFORMATION AND REGISTRATION:

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www.lotassa.org

"LOTASSA"

LOTus **A**daptation and
Sustainability in **S**outh **A**merica

Bridging Genomics and
Agrosystems Management

International Workshop

Integrating Genomics
into Plant Breeding

October 25 and 26, 2007
Porto Alegre, Brazil

Address: Blue Tree Millenium Flat
Av. Borges de Medeiros, 3120,
Praia de Belas - Porto Alegre, RS

ORGANIZADORES DEL WORKSHOP:
WORKSHOP ORGANIZERS:

DR. MIGUEL DALL' AGNOLL (UFRGS)
DR. ENILSON SACCOL DA SÁ (UFRGS)
DR. EMILIO RUZ (PROCISUR)

SECRETARÍA DEL WORKSHOP:
WORKSHOP SECRETARIAT:

GLADYS FERNÁNDEZ (PROCISUR)

ENTIDADES COLABORADORAS:
UNDER THE SPONSORSHIP OF:

SIXTH FRAMEWORK PROGRAMME

LOTASSA

LOTUS ADAPTATION
AND SUSTAINABILITY
IN SOUTH AMERICA

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FEDERAL DO
RIO GRANDE
DO SUL

PROCISUR

PROGRAMA COOPERATIVO PARA EL
DESARROLLO TECNOLOGICO
AGROALIMENTARIO Y AGROINDUSTRIAL
DEL CONO SUR

Programme

Thursday, October 25

The LOTASSA Project

Chairperson: Mónica Rebuffo (Uruguay)

09:00-9:30 **Juan Sanjuán** (Spain)
General objectives, components, integration and perspectives.

Section 1: Model Legumes

Chairpersons: Mariana Melchiorre (Argentina) and Peter Palove-Balang (Slovakia)

09:30-10:00 **Niels Sandal** (Denmark)
Utility of genetic and genomic resources from *L. japonicus* to cultivated species of *Lotus*.

10:00-10:30 **Michael Udvardi** (USA)
Genomics from *Medicago truncatula*: advances and potential use in other species.

11:00-11:30 **Ryo Akashi** (Japan)
L. japonicus genetic and genomic resources in Japan.

11:30-12:00 **Marcio Moretzsohn** (Brazil)
Can legume synteny be useful in guiding the introgression of wild genes into cultivated peanut?

12:00-12:30 Discussion

14:00-14:30 **Michael Udvardi** (USA)
Postgenomics tools to study plant growth and development (metabolomics, proteomics and transcriptomics).

14:30-15:00 **Manuel Becana** (Spain)
Oxidative stress as response to salinity and aluminum.

15:00-15:30 **Antonio Márquez** (Spain)
Nitrogen metabolism in legumes.

16:00-16:30 **Antonio Márquez** (Spain) and **Jorge Monza** (Uruguay)
Physiological responses of *Lotus* to stresses.

16:30-17:00 Discussion

Friday, October 26

Section 2: Biotechnology and Plant Breeding

Chairpersons: Manuel Ortega (Chile) and Diego Sánchez (Gemany)

08:0-08:30	**Heathcliffe Riday** (USA) Marker assisted selection in legumes.
08:30-09:00	**Merixtell Antolin Llovera** (Germany) Tilling: examples of utilization in plant breeding.
09:00-09:30	**Mónica Rebuffo** (Uruguay) Breeding temperate legumes: advances and challenges.
10:00-10:30	**Nora Altier** (Uruguay) Diseases of forage legumes: advances and prospects of research on management strategies.
10:30-11:00	**Federico Costa Beber Vieira** (Brazil) Effect of crop systems and nitrogen application upon soil acidification.
11:00-11:30	Discussion

Section 3: Microbiology

Chairpersons: Meritxell Antolin Llovera (Germany) and Fernando Pieckenstain (Argentina)

13:00-13:30	**Carlos Labandera** (Uruguay) Inoculants on forages: practical aspects and actual situation of their usage in relation to other countries.
13:30-14:00	**Jasmin Gossmann** (Germany) Signal transduction in plant-microbe interactions.
14:00-14:30	**Fátima Moreira** (Brazil) Soil biodiversity: genetic resources to enhance nitrogen fixation.

Section 4: General Perspectives

Chairpersons: Juan Sanjuán (Spain) and Mónica Rebuffo (Uruguay)

15:00-15:30 **Walter Ayala** (Uruguay)
Forage and Animal Production in the region.

15:30-16:00 **Carlos Foderé** (Uruguay)
Seed industry requirements.

16:00-16:30 **Martín Lage** (Uruguay)
Requirements from the inoculants industries.

16:30-17:00 **Carlos Nabinger** (Brazil)
Animal production: an integrated point of view.

17:00-17:30 Discussion

Contents

International workshop

Integrating genomics into plant breeding.

Lotus Newsletter (2007) Volume 37 (3), 91.

Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

LOTASSA: first glance of model to cultivated forage legumes

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[*] *Corresponding author*

Due to the current pressure imposed by the expansion of intensive crop systems, pasture areas are banished to marginal, low fertility soils. Further, abiotic stress tolerance in crop and pasture plants is becoming increasingly important with global climate changes and other consequences of anthropogenic activities, such as the global increase in desertification, soil salinity, etc. As a consequence, pastures are grown increasingly at the limit of their adaptation, in areas where the ability to survive periodic (i.e. drought, flooding) or continuous (i.e. pH stress, salinity) environmental stresses become an essential characteristic for success. One of the main challenges for breeders will be the production of superior germplasm that allows to maintain, if not enhance the current agricultural yields under increasingly adverse environmental conditions. Consequently, breeding programmes that aim to systematically improve general abiotic stress tolerance in plants are of prime importance for the sustainability of food production. The implementation of breeding programs, assisted by the latest knowledge and technologies, towards the development of improved cultivars adapted to environmental stresses, appears as a feasible strategy to increase the productivity of current grazing areas, essential for enhancing livestock production. Compared with *Trifolium* or *Medicago*, *Lotus* species have a number of advantages that make them successful in the Southern Cone, like better adaptability to low P availability and low pH soils, as well as excellent animal performance in grazing systems. Breeding for tolerance to abiotic stresses in *Lotus* spp. of agronomic interest has been restricted by the reproduction system and the complexity of the physiological and metabolic responses involved, as well as the symbiotic specificity. Although progress could be made in developing stress-tolerant cultivars of cultivated species using conventional breeding, the pace of this progress may be too slow to meet the growing needs of improving natural pastures. Given the close genetic relatedness among *Lotus* species, the LOTASSA project aims to promote the existing genetic and genomic resources for the model species *Lotus japonicus* to improve adaptation and sustainable production of agriculturally important *Lotus* species in environmentally constrained soils in South America. The innovation of LOTASSA is the joint research on plant and rhizobia of model and cultivated *Lotus* species by European and South American Institutes on the genetic and metabolic bases of the physiological processes involved in salt, water and Al stresses.

Lotus Newsletter (2007) Volume 37 (3), 92.

Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Utility of genetic and genomic resources from *L. japonicus* to cultivated species of *Lotus*

NIELS SANDAL*, NIELS JØRGENSEN, SVEND DAM, GITTE NAUTROP, JAKOB FREDSLUND, BIRGIT K. HOUGAARD, SIMONA RADUTOIU, LEILA TIRICHINE, LENE H. MADSEN, ESBEN B. MADSEN, KEISUKE YOKOTA, PALOMA ROMERO, ANNA JURKIEWICH, ANITA ALBREKTSEN, ESBEN M.H. QUISTGAARD, EIGO FUKAI, JENS STOUGAARD

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* *Corresponding author*

The molecular genetics of *Lotus* is focused on three diploid species: *Lotus japonicus, Lotus filicaulis* and *Lotus burttii*. In addition to the inbred germplasm of these species a resource of recombinant inbred lines has also developed from *L. filicaulis* x *L. japonicus* ecotype Gifu, from *L. japonicus* ecotype Gifu x *L. burttii* and from *L. japonicus* ecotype Gifu x *L. japonicus* ecotype MG20. In parallel several methods for genetic analysis of gene function have been established within the *Lotus* community. Insertion mutagenesis with T-DNA, transposable elements and retrotransposons as well as EMS mutagenesis have all been used in *Lotus japonicus*. To enable map-based cloning genetic maps are constructed and different methods for positional cloning of symbiotic loci are currently applied in order to clone genes involved in nodule initiation, nodule function as well as autoregulation (Tirichine *et al*., 2007). At Kazusa DNA Research Institute, Japan the genome of the model *Lotus japonicus* is under sequencing and the complete sequence of substantial parts of the genome is already available in public databases. The sequencing program is focused on gene rich regions and an approach using seed points anchoring sequences onto the genetic map has been developed. Taking advantage of the available genome and EST sequences a proteomic program has been initiated on seed proteins and a transcriptome analysis based on Affymetrix will soon be available. A summary of the structural and functional genomics within the *Lotus* community and the future perspectives will be given together with a discussion of the possibilities for transfer of information into cultivated legumes (Fredslund *et al*., 2006).

References

TIRICHINE, L., SANDAL, N., MADSEN, L.H., RADUTOIU, S., ALBREKTSEN, A.S., SATO, S., ASAMIZU, E., TABATA, S., STOUGAARD, J. 2007. A Gain-of-Function Mutation in a Cytokinin Receptor Triggers Spontaneous Root Nodule Organogenesis. *Science*, **315**, 104-107.

FREDSLUND J., MADSEN L.H., HOUGAARD B.K., NIELSEN A.M, BERTIOLI D., SANDAL N., STOUGAARD J. and SCHAUSER L. 2006. A general strategy for the development of anchor markers for comparative genomics in plants. *BMC Genomics*, **7**, 207.

Lotus Newsletter (2007) Volume 37 (3), 93.

Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Genomics of *Medicago truncatula*: advances and potential use in other species

MICHAEL UDVARDI[1], VAGNER BENEDITO[1], JIYI ZHANG[1], JEREMY MURRAY[1], IVONE TORREZ-JEREZ[1], RUJIN CHEN[1], MILLION TADEGE[1], KIRAN MYSORE[1], JIANGQI WEN[1], JI HE[1], PATRICK ZHAO[1], YUHONG TANG[1], CHRISTOPHER D. TOWN[2], GEORG WEILLER[3], KLEMENTINA KAKAR[4], MAREN WANDREY[4], ZENG-YU WANG[1], and RICHARD A. DIXON[1]

[1]*The Samuel Roberts Noble Foundation, USA*

[2]*The J. Craig Venter Institute, USA*

[3]*The Australian National University, Australia*

[4]*Max Planck Institute of Molecular Plant Physiology, Germany*

Legumes are second only to grasses in importance to humans as a source of food, feed for livestock, and raw materials for industry. The Noble Foundation is committed to the improvement of legumes for pastures. This includes enhancing the nutritional value of legumes as well as increasing the tolerance or resistance of legumes to abiotic and biotic stress. Although alfalfa, *Medicago sativa*, is the most important forage legume in the USA, it is a poor model for genetic and genomic research. However, its close relative, *Medicago truncatula* (or simply Medicago), which has a comparatively small diploid genome is an ideal model for legume genomics. The Noble Foundation has played a leading role in the establishment of Medicago as a model and is using it to identify target genes for legume breeding programs. This talk will review our work on the development and use of tools for Medicago functional genomics, which include a gene expression atlas for *Medicago truncatula*, platforms for high-throughput qRT-PCR analysis of transcription factor and miRNA genes, and fast neutron deletion and *Tnt1* transposon insertion mutant populations. A recent example of how discoveries in *Medicago truncatula* can translate to improvements in alfalfa is the production of low-lignin alfalfa with improved digestibility and nutritive value for animals, details of which will be presented at the meeting.

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Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Lotus japonicus genetic and genomic resources in Japan

RYO AKASHI*[1], MASATSUGU HASHIGUCHI[1], TAKAHIRO GONDO[1], JUN ABE[2], TOSHIO AOKI[3], TATSUYA SAKAI[4], TOYOAKI ANAI[5], AKIHIRO SUZUKI[5]

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Japanese trefoil (*Lotus japonicus*) is a wild perennial plant with a small genome and a short life cycle. This plant is expected to play a role as the model organism of leguminous plants, which include important crop plants such as soybean (*Glycine max*). Legume Base, a resource center for *L. japonicus* and *G. max*, was established in April 2004. The scope of Legume Base is the collection, development and conservation of the genetic resources of *L. japonicus* and *G. max* and the distribution of the material for utilization by the research community. DNA resources including genomic DNA clones will be also available through Legume Base web site (http://www.legumebase.agr.miyazaki-u.ac.jp). Legume Base is supported by the National BioResource Project (NBRP) of Japan. The core facility of Legume Base is Miyazaki University and the sub facility is Hokkaido University. Some parts of the distribution work is carried out by the following facilities on commission: Nihon University, RIKEN Yokohama Institute and Saga University. The resources listed below in **BOLD** are now available.

Lotus japonicus

1. **Experimental lines** (Miyakojima MG-20, Gifu B-129, L. burttii B-303)
2. **Wild accession lines** (collected throughout Japan)
3. **LjMG RI lines** (RI lines between Miyakojima MG-20 and Gifu B-129)
4. Activation tag lines
5. **EMS mutants**
6. **Root culture system** (Super Roots isolated from L. corniculatus)
7. **DNA resource** (TAC, BAC, cDNA)

Glycine max

1. Cultivated accession lines
2. **Wild accession lines** (collected throughout Japan)
3. **RI lines** (RI lines between Misuzudaizu and Moshidou Gong 503)
4. **RI lines** (RI lines between Tokei 780 and Hidaka 4)
5. **X-ray Mutants** (Fatty acid composition)

Legume Base URL; http://www.legumebase.agr.miyazaki-u.ac.jp
Contacting us (E-mail); legume@agr.miyazaki-u.ac.jp

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Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Can legume synteny be useful in guiding the introgression of wild genes into cultivated peanut?

MÁRCIO MORETZSOHN[1], SORAYA LEAL BERTIOLI[1], PATRICIA GUIMARÃES[1], LENE MADSEN[2], JAKOB FREDSLUND[2], BIRGIT HOUGAARD[2], LEIF SCHAUSER[2], NIELS SANDAL[2], JENS STOUGAARD[2], SATOSHI TABATA[3] and DAVID BERTIOLI[4*]

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[*] *Corresponding author*

Most agriculturally important legumes fall within two sub-clades of the Papilionoids. The phaseoloids clade is an essentially tropical group that includes bean, cowpea, soya and pigeon pea. The galegoids, are mostly temperate, and include clover, pea, lentil, field bean, chickpea, and *Medicago* and *Lotus*. However, peanut (*Arachis hypogaea* L.) falls in the more basal, mostly tropical, Dalbergioid clade. Cultivated peanut is an allotetraploid of recent origin, with genome type AABB. A severe genetic bottle-neck was imposed at the species origin, via hybridisation of two wild diploid species followed by a spontaneous chromosome duplication. This led to a low genetic diversity which, coupled with the complexity of tetraploid genetics, has constrained the advances in genetics necessary for modern breeding. Under the framework of the Generation Challenge Program we have worked to overcome these limitations. Firstly, we dissected the tetraploid genetics of peanut by constructing two diploid maps: one for the AA (Moretzsohn *et al.*, 2005), and one for the BB genome. Secondly we are constructing tetraploid maps, using synthetic amphidiploids. For markers, we used microsatellites, because they are codominant and polymorphic, have good transferability between species, and are ideal for breeders. We have used the same markers for all maps, thus generating a framework for map comparison and the mapping of QTLs in different genetic backgrounds. Clearly, the utility of the reference map, based on the AA-genome map, would be greatly enhanced if it could be used to access the near-complete genome sequences of *Lotus* and *Medicago*, in a way that would enhance our understanding of the *Arachis* genome. With this aim, we placed more than 80 legume anchor markers (Fredslund *et al.*, 2006) on the AA-genome map and analyzed the synteny between *Arachis* and the model legumes. The main affinities of nine of the ten *Arachis* linkage groups and model legume chromosomes were identified. Some substantial regions of marker co-linearity are evident. The data indicates that the last whole genome duplication seems to have predated the divergence of *Arachis* from the Galegoids and Phaseoloids. The inclusion of *Arachis* within a single genetic system for the legumes appears to be feasible. This should help in the characterization of the *Arachis* genome, and the implementation of molecular breeding for this crop.

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FREDSLUND J., MADSEN L.H., HOUGAARD B.K., NIELSEN A.M., BERTIOLI D., SANDAL N., STOUGAARD J., SCHAUSER L. 2006. A general strategy for the development of anchor markers for comparative genomics in plants. *BMC Genomics*, **7**:207.

MORETZSOHN M.C., LEOI L., PROITE K., GUIMARÃES P.M., LEAL- BERTIOLI S.C.M., GIMENES M.A., MARTINS W.S., VALLS J.F.M., GRATTAPAGLIA D., BERTIOLI D.J. 2005. A microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). *Theoretical and Applied Genetics*, **111**:1060-1071.

Lotus Newsletter (2007) Volume 37 (3), 97.

Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Postgenomic tools for *Lotus japonicus*: Application to abiotic stress research

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Lotus japonicus is an ideal model species for legume genetics, genomics, and functional genomics research. Large-scale EST and genome sequencing has facilitated rapid progress in the areas of transcriptomics and proteomics, which together with tools for metabolomics are transforming the way experimental biology is performed. To illustrate this transformation, we present the results of a recent study of plant responses to non-lethal long-term salinity. Ionomic profiling by inductively coupled plasma-atomic emission spectrometry (ICP-AES) revealed salt stress-induced reductions in potassium, phosphorus, sulphur, zinc and molybdenum. Microarray profiling using the Lotus Genechip® allowed the identification of 912 genes differentially expressed during salt acclimation. Gas chromatography-mass spectrometry (GC/EI-TOF-MS)-based metabolite profiling identified 147 differentially accumulated soluble metabolites, indicating a change of metabolic phenotype upon salt acclimation. Metabolic changes were characterised by a general increase in the steady-state levels of many amino acids, sugars and polyols with a concurrent decrease in most organic acids. Transcript and metabolite changes exhibited a stress-dose dependent response within the covered range of NaCl concentrations, although threshold and plateau behaviors were observed. The combined observations suggest a successive and increasingly global requirement for the reprogramming of gene expression and metabolic pathways to maintain ionic and osmotic homeostasis during salt stress.

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Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Oxidative stress as a response to salinity and aluminum

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Reactive oxygen species (ROS), such as the superoxide radicals and hydrogen peroxide, are formed in processes involving electron transfer. In plants, ROS are generated in many cellular compartments, especially in the chloroplasts, mitochondria, and peroxisomes. In legume root nodules, ROS are also produced at high rates during bacteroid respiration. Under physiological conditions, plants are endowed with a great variety and quantity of antioxidant enzymes and metabolites to keep ROS concentrations under tight control. This is important to avoid the potential toxicity of ROS while allowing them to perform useful functions in growth, development and stress signaling. However, in plants exposed to several types of abiotic and biotic stress, an excess of ROS production and/or a decrease in antioxidant protection may lead to oxidative stress. This situation can be diagnosed by the accumulation in plant cells of lipid peroxides and oxidatively-modified proteins, among other biochemical markers. In this talk, I will review what is known on the ensuing of oxidative stress in plants exposed to salt and aluminum stress, with an emphasis on legumes. A summary of main conclusions follows. *Salt stress* causes reduction in plant growth and photosynthesis, and increases of Na and proline (but decreases in K and Ca) in plant tissues. Salinity also leads to increases in the activities of antioxidant enzymes [superoxide dismutases (SOD), ascorbate-glutathione pathway], in the production of ROS, and in the contents of lipid peroxides and oxidized proteins. It is concluded that the there is an integrative effect of salt stress (NaCl concentration x duration of treatment), probably related to the Na concentration in tissues. It is also propose that this parameter, together with proline, lipid peroxidation, and the antioxidant enzymes SOD and dehydroascorbate reductase, are good markers for salt stress tolerance/sensitivity. *Aluminum* is a nonessential metal that causes toxicity to plants in acid soils, where the metal is highly mobile. Very little is known about the effects of Al on the antioxidant systems of plants, and most data are restricted to roots. Also, there are variable results depending on the experimental protocol, plant species, and plant age. A main and general effect of Al (at micromolar levels) is the rapid inhibition of root growth, but treatment of plants with Al also causes an accumulation of lipid peroxides in tissues, which is indicative of oxidative stress. However, much more work will be needed to ascertain whether the toxicity of Al is due, at least in part, to oxidative damage of cellular components and to determine the effects of this metal on antioxidant systems and ROS production.

Lotus Newsletter (2007) Volume 37 (3), 99-101.

Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Nitrogen metabolism in *Lotus japonicus* and the relationship with drought stress

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In our laboratories we carry out research on nitrogen assimilation in *Lotus* plants and the possible relationships with drought stress situations that become a likely cause of the loss of these forage plants when they are cultivated (Díaz *et al.*, 2005a; 2005b). Some of our research has been conducted with the model legume *Lotus japonicus,* while other research was done on cultivated species. *Lotus japonicus* plants are able to use both nitrate and ammonium as inorganic nitrogen sources for ulterior assimilation, or, alternatively, they can also use atmospheric dinitrogen through *Mesorhizobium loti* symbiosis. Primary nitrate assimilation takes place predominantly in the roots of the plant, being strongly dependent on the age and limitation of space for root growth (Orea *et al.,* 2001; Pajuelo *et al.*, 2002*).* Attempts of genetic manipulation of root-shoot partitioning of nitrate assimilation, either by increasing external nitrogen availability (Orea *et al*., 2005a), or using a transgenic approach (Orea *et al*., 2005b), were not able to shift this partitioning to the aerial part of the plant, thus suggesting the existence of ecophysiological adaptations for a preferential use of external nitrogen in the root (Márquez *et al*., 2005). This situation makes crucially important the mobilization of nitrogen from roots to shoots of the plant, particularly with regard to asparagine metabolism. On the other hand, in our laboratory we have also recently shown the importance for this plant of other forms of secondary nitrogen assimilation such as reassimilation of ammonium released by photorespiration. We have used a mutagenesis approach to demonstrate the essentiality of plastidic glutamine synthetase in this process. However, this was not the case for primary ammonium assimilation, a process which can rely basically on cytosolic glutamine synthetase (Orea *et al*., 2002; Betti *et al*; 2006). The use of these mutants enabled to show that there is also some influence of photorespiration on the level of different ammonium transporters (D'Apuzzo *et al*., 2004) as well as nodule development and starch metabolism (García-Calderón *et al*., 2007). Nitrogen metabolism in *Lotus* plants shows also a strong connection with drought stress situations, mainly through the biosynthesis of proline, which becomes a very nice marker of osmotic stress situations in this plant (Díaz *et al*., 2005 b,c). Proline metabolism is greatly influenced by the type of nitrogen nutrition provided to the plant (Díaz *et al.*, 2005c). At present we are also investigating the possible interconnection between photorespiration and drought stress situations in these plants.

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Abstract, Workshop Porto Alegre, Brazil – 25-26 October 2007

Marker assisted selection in legumes

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Marker assisted selection (MAS) has begun to be implemented in some self-pollinating crops such as wheat. The promise of MAS has been on the breeding horizon for over two decades now. With the advent of the *Medicago truncatula* and *Lotus japonicus* genome sequences; numerous SSR-mapped markers in *M. truncatula*, *Medicago sativa*, *Trifolium pratense*, and *Trifolium repense*; and various EST projects in forage legume species, a good genomic infrastructure has been developed. In this context, an exploratory integrated QTL discovery and selection scheme has been implemented in *T. pratense*. *T. pratense* (red clover) is a diploid outbreed species with limited breeding resources due to smaller seed market share, with biomass yield and plant persistence being the overwhelming traits of breeding interest. This makes red clover an ideal test case for determining MAS feasibility, both scientifically and economically. An analysis of a recurrent halfsib selection scheme overlaid with a simultaneous recurrent linkage disequilibrium (LD) QTL discovery and MAS scheme shows substantial increased genetic gain per cycle could be achieved for traits, such as persistence, that are difficult to phenotype and highly quantitative. However, consideration of molecular technology costs, along with correspondingly reduced field evaluation expenditures, makes the genetic gains in MAS selection schemes much more marginal. One of the most powerful uses of molecular markers in a halfsib recurrent selection scheme would be to increase parental control by identifying paternity for truncation selection within field selected halfsib lines. LD QTL discovery methods fit very nicely into recurrent halfsib selection schemes, since almost any marker associations with the trait (real or population structure induced) can be used for either increased parental control or QTL enrichment. Reducing genotyping costs to \$0.03 USD or less per marker per genotype would make MAS very competitive in red clover persistence selection. Even at current cost estimates of over \$0.30 USD per marker per genotype, MAS could play a profitable role in paternity identification and selection schemes.

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TILLING: Examples of utilization in plant breeding

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Targeting Induced Local Lesions IN Genomes (TILLING), first published from McCallum *et al.* (2000a), is a reverse genetics tool that combines an ethyl mathanesulfonate (EMS)-induced mutagenesis with the identification of novel genetic variation (reviewed in McCallum *et al.*, 2000b; Colbert *et al.*, 2001). This method generates a wide range of mutant alleles, is automatable and is applicable to any organism that can be chemically mutagenised. This technique was developed in *Arabidopsis thaliana* (McCallum *et al.*, 2000a) and also works in other plant species, including crops (reviewed in McCallum *et al.*, 2000b; Colbert *et al.*, 2001). TILLING is a non-transgenic tool to obtain crop species with advantageous characteristics as demonstrated in wheat (Slade *et al.*, 2005). Using the same detectable technology, naturally evolved polymorphisms can be identified between ecotypes (Eco-TILLING) (Comai *et al.*, 2004; Gilchrist *et al.*, 2006; Nieto *et al.*, 2007). Therefore, genes related to biotic or abiotic responses are potential targets for Eco-TILLING. A TILLING platform has been established for the model legume *Lotus japonicus* (http://www.lotusjaponicus.org/tillingpages/Homepage.htm) (Perry *et al.*, 2003).This facility is being used in the framework of the LOTASSA project with the objective of obtaining *Lotus* plants more tolerant to environmental constrains. In conclusion, TILLING and/or Eco-TILLING can be applied in plant breeding for detection of alleles associated with agronomically important traits.

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Breeding temperate legumes: advances and challenges

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Conventional breeding is an efficient tool with factors with low environmental effect, where the phenotype expression is closer to the genotype. On the other hand, selection for abiotic stresses involves complex characteristics restricting progress of conventional breeding programs. The study of the responses of several perennial legumes to selection for water and saline stress, Al tolerance and P availability is developed in the frame of the Projects LOTASSA (FP6-2003-INCO-DEV2 PL-517617) and LESIS (FTG-787/2005). Their approach involves the characterization and selection of naturalized populations for the identification of superior germplasm, as well as their metabolic and molecular analysis in order to develop strategies that could spead conventional breeding in the future. The presentation describes the approach and first results. The physiological responses of the main cultivated species to Al, saline and water stress are evaluated. Divergent selection is carried out in *Lotus corniculatus* (*Lc*) and diploid *L. uliginosus* (=*L. pedunculatus*) (*Lu*) for tolerance to water stress and Al tolerance, and the development of contrasting populations for root characteristics (root branching and density), the characterization of natural populations of *L. glaber* (=*L. tenuis*), *Trifolium repens* (*Tr*) and *T. pratense* (*Tp*) for water stress, *L. glaber* for saline stress. Chilean and Uruguayan naturalized populations of *Lc, Tr* and *Tp* are evaluated for water stress and prevailing diseases. Results show that there is genetic variability within the *Lc, Lu* and *Lg* germplasm for Al tolerance, water and saline stress, respectively, to be used in the selection process. *Lc* ultivar San Gabriel (Uruguay) was more sensitive to Al toxicity than Brasilian germplasm; the membrane potential in root cortex cells remains unchanged between pH 4.4 and 6 for the most tolerant Brasilian selections. Although saline stress stunted foliar and root growth, the evaluation at young stages (40 days) showed *Lg* entries collected in saline soils of Buenos Aires Province (Argentine) that produced 50% more forage than less adapted germplasm. There are differential responses of Chilean *Lg* accessions to water stress in leaf rate appearance, stem elongation and stem dry weight. Water stress (40% field capacity) was imposed for six months to *Lc* cultivar San Gabriel and INIA Draco. Contrasting phenotypes were identified through regrowth production (2.15 and 4.13 g/plant of fresh weight for sensitive and tolerant selections, respectively). Differences between sensitive and tolerant selections were larger for *Lu* (2.96 and 7.72 g FW/plant). Proline is one of the metabolite that shows high consistency with the response to water stress in several species of *Lotus* as well as in *Tp*. The isotopic discrimination (Δ13C/12C) has a good correlation with water use efficiency for the cultivated *Lotus* species.

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Diseases of forage legumes: advances and prospects of research on management strategies

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Forage legumes are essential for an efficient animal-based agriculture. They provide high quality feed for livestock and are a key component for sustainability of crop-pasture rotations. Diseases are a major cause of weakened establishment, premature stand decline and reduced productivity in most temperate forage legumes. The perennial strategy of crown-forming species like alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and red clover (*Trifolium pratense* L.), relies on the success of individual plants to develop and maintain a healthy crown and root system along the stand life. The interaction of diverse biotic and abiotic factors does likely produce a cumulative stress load and pose a threat to the long-term performance of forage legumes. My research program is focused on developing management strategies for minimizing the impact of diseases on forage legume establishment, production and persistence. Current research explores the biological control of *Pythium* seedling diseases using native fluorescent *Pseudomonas* (Bajsa *et al.*, 2005; Pérez *et al.*, 2001). Several strains with enhanced disease suppressing and plant growth promoting abilities have been selected to develop bacterial inoculants (De La Fuente *et al.*, 2002; Quagliotto *et al.*, 2004; Yanes *et al.*, 2005). I work in close collaboration with the breeding programs in the development of new varieties with improved disease resistance (Altier *et al.*, 2000; Real and Altier, 2005). My research facilitates the development of new techniques and standardized tests to characterize germplasm and to assist in the identification of host plant resistance (Altier and Thies, 1995). Other projects involve interdisciplinary approaches to understand the ecology of forage legume microbes and disease epidemiology as influenced by agricultural and crop management practices (Altier, 2003; Bao *et al.*, 2005). Results of research on *Fusarium* crown and root disease complex have been recently reported (Altier and Groth, 2005; Altier and Kinkel, 2005). This knowledge is essential to establish the basis for efficient and durable means of disease management.

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Effect of crop systems and nitrogen application upon soil acidification

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Important advances were obtained in the understanding of soil acidification due to agriculture/husbandry in the last years. The work of some scientists, mainly after 1980`s, deserves special consideration, taking into account their contributions to the comprehension of the main factors and processes involved in soil acidification (Helyar and Porter, 1989). However, much is still lacking in getting a holistic view about soil acidification when all factors of environment are present and, most important, in what we can do for diminish soil acidification and make sustainable the land exploitation. The main challenge is to understand exactly how and why it happens in order to manage soil in such a way to minimize the rates of soil acidification, devising strategies to reduce rates of acidification other than by applying lime. As important as to refine the current knowledge is to diffuse this knowledge to people that deal with soil acidification. In agriculture systems, the C and N cycles are the main factors of soil acidification. In the C cycle, as plants develop and uptake nutrients - mostly as cation forms - the root system exudates protons in order to maintain the electrical neutrality of its cells, leading to the transient generation of acidity in the soil and, concomitantly, the plant material becomes potentially alkaline. In a closed system, when there is no input or output of material, the alkalinity present in the plant material is equal to the soil acidity and there is neutralization after plant death. However, when part of the alkaline material is removed from the system as grains, hay, silage, or as animal products, there is not enough alkalinity to neutralize all acidity generated in the soil, resulting in net soil acidification. In the N cycle, legume species and mineral N can contribute with soil acidification mainly due to increase in nitrate leaching and by increasing the exportation of alkaline plant material. In addition, each mineral N fertilizer has different reaction in the soil and, depending on what source is applied, mineral N can promote soil acidification even when no N is lost due to nitrate leaching. In this context, vegetation can affect soil acidification through variations in N fixation capacity, in the ability to absorb and recycle nutrients from and in the soil profile, in its ability to minimize nutrient leaching through effects on water use and nutrient ‘stripping’ from the leached water, in concentrations of anions – amount of alkali lost in removal of plant material, build-up in organic matter, and its effect on erosion of fertile topsoil. In a long-term no-till experiment without liming, Vieira (2007) found higher rates of soil acidification in legume-based than in grass-based crop systems, and soil acidification was exacerbated by N-urea application. The interesting fact is that, in both cases, the increase in soil acidification was mainly due to the N effect on the increase in grain yield, and not due to increases in nitrate leaching. The main strategies to diminish soil acidification by management are to improve the balance between N supply and plant requirement/uptake; choose mineral N fertilizers that have more alkaline reaction;

keep live plants cycling and absorbing nutrients; in grazing pastures, cultivate grass species together with legume species, choosing preferentially species with deep and abundant root system; develop strategies to conciliate low soil pH and adequate plant development; search for Al-tolerant genotypes; insert Al-tolerance characteristics in other genotypes; improve soil organic matter contents in order to maximize Al complexation.

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Forage legume inoculation

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This paper summarized the antecedents and activities in biological nitrogen fixation in forage legumes in the Soils Microbiology Department – Land and Water Division – Renewable Natural Resources General Direction – Ministry of Livestock, Agriculture and Fisheries – Uruguay, discusses the main technological adoption factors and compare the use of inoculants in different countries. The Department was created in 1960 in the Plan Agropecuario to study the importance of legume inoculation in the establishment, productivity and persistence of forage legumes in the country. The Project was initiated with a rhizobia strain selection programme and supports (peat) for inoculants production. The National Inoculant Industry was established as a result of this first effort, as well as the legal frame to guarantee inoculants quality. Due to this reason, a very close functional tie was created between this Department, the Inoculant Industry and the Farmers. The role of the Plan Agropecuario in relation to the spreading/application of this technology in the farms is pointed out, as well as the identification of the limiting factors for symbiosis, which allowed to define a research program close related to the productive sector demands. During the 1980s the use of sterile peat in inoculants was spread and the requirement levels were enlarged to 2 and 1x10-9 rhizobia/gr of product at marketing and expiration time respectively. A very important interdisciplinary and inter-institutional relationship has been established since 1990, through research projects with external financing and grade thesis with university students. This relationship enabled the extension of activities to study the agronomic potential of other plant growth promoting micro-organisms with emphasis in gramineous plants. A wide range of activities and projects with emphasis in BNF by the *Rhizobium*-legume symbiosis is being developed since 2000, but keeping increasing efforts on other systems. The definition of a research policy based on coordinated projects at national and international levels is emphasized. At the same time, new inoculants formulations (liquid inoculants) and inoculation techniques are being developed and validated with the industry support, especially in response to the increasing booming of soybean cultivation in the country. Forage legume inoculation is a very wide practice in Uruguay with adoption percentages close to 100%. This situation represents for the agricultural sector and the country, an annual saving of 300 million dollars for partial substitution of nitrogen fertilizers, which it would have been necessary to import to reach actual productivity levels of pastures and crops. Even though, these systems would not be sustainable in time, as the mechanisms of biologic nitrogen fixation is ecologically more stable and uses a natural renewable resource.

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Soil biodiversity: genetic resources to enhance nitrogen fixation in agriculture and forestry

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Leguminosae is the third largest family of flowering plants. Although about 75% of the extant 20.000 Leguminosae species are unknown regarding their ability to establish a mutualistic symbiosis with nitrogen fixing bacteria, near 3.500 species around the world are already known to present this characteristic. Leguminosae is an important family in Brazilian ecosystems both in diversity and density. It is estimated around 2.000 native species. Hence, they represent an important source for new species and strains of Leguminosae nodulating bacteria (LNB) to be explored and to enhance symbiotic nitrogen fixation. Until now, 55 LNB species were described. Among them, five were described considering Brazilian native strains (*Rhizobium tropici*, *Mesorhizobium plurifarium*, *Sinorhizobium adherens*, *Azorhizobium doebereinerae*, *Burkholderia mimosarum*). Results of intensive surveys on soil LNB diversity indicate these figures can be much higher. Bacterial species/strains and plant species vary regarding the establishment of their symbiosis from highly specific, i.e., they are able to form symbiosis with just a narrow range of partner species/strains, to highly promiscuous, when they are able to establish symbiosis with a large range of partners. Regarding functionality, strains efficiency usually exhibit a large range even within the same species in symbiosis with a given host species. *Azohizobium doebereinerae* & *Sesbania virgata* specific symbiosis seems to be an exception, as all strains tested until now were highly efficient. Siratro (*Macroptilium atropurpureum*), common beans (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) are very well known promiscuous hosts establishing symbiosis with a large number of species and genera which vary a lot regarding nitrogen fixation efficiency. These plant species has been used as traps for the study of LNB diversity and the last two are important crops which could better benefit from symbiotic nitrogen fixation. Specificity/promiscuity, efficiency and adaptation to environment conditions must be considered for both strain selection and inoculant efficiency. *Lotus* spp. establish symbiosis the high promiscuous strain *Rhizobium* spp. NRG234, *Rhizobium etli Bradyrhizobium* sp. and with *Mesorhizobium loti*. Strain *M. loti* MAFF 303099 was the first LNB genome already sequenced. Comparison of its genome to other LNB genomes revealed that genes and regions involved in symbiosis and nitrogen fixation exhibited a high diversity in their organization. Efficient strains have been selected and recommended for inoculant production of 109 leguminous species, including those used for multi-purpose trees, grains, forage (e.g. *Lotus* spp.), and green manure. According to MAPA (Ministério da Agricultura, Pecuária e Abastecimento), 26,4 million doses of inoculant were produced and commercialised in 2003, the vast majority, i.e. 99%, being for

soybeans. Thus, the remaining 1% was for the other 108 species, indicating diffusion of this biotechnology need to be improved. BNF in soybeans was estimated in 2,60 million Mg N in 2006/2007. This figure is greater than the 2,34 million Mg used in 2006/2007 by all crops in the same year, from which 64 % were imported. If inoculants were applied intensively to the other legume species, direct and indirect (e.g. green manure to non-legumes) benefits of nitrogen fixation could enhance crop productivity in an environmentally sound way. RELARE (Rede de laboratórios para recomendação, padronização e difusão da tecnologia de inoculantes microbiológicos de interesse agrícola) is a laboratory network responsible for recommendation, standardization and diffusion of inoculant technology relevant to agriculture. Usually one to four strains are recommended as inoculant to each one of the 109 plant species without considering yet the large variability of climatic and edaphic conditions in Brazil. Although, a great progress was made until now, it is obvious, much more is needed to be done in order the important biological process of nitrogen fixation can totally replace agriculture and forestry requirements. Improving soil biodiversity knowledge implies not only enhancing genetic resources conservation but also better ecology knowledge for improvement of management practices, including inoculation.

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Forage and animal production in the region

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Natural grasslands represent a large area of "campos" ecosystem in the southern of Brazil, Uruguay and northeast of Argentina between 30 and 38 degrees of latitude (Soriano, 1988), supporting beef and sheep meat, wool and dairy production. Forage production is low in quantity and quality, varying between seasons and years (Bermúdez and Ayala, 2005), associated to rainfall regimes (Berretta *et al*., 1999). Warm season grasses (C_4 species) are predominant with a low proportion of legumes. Beef cattle production in extensive systems is characterized by an advanced heifer mating age, low calving rate, low calf liveweight gains, advanced slaughter age, low extraction rate and annual productivity of 65 kg of liveweight/ha/year (Berretta *et al*., 1999). Sheep production shows a hogget mating age around 2.5 years with high lamb mortality (Berretta *et al*., 1999). Improved "campos", including legumes and phosphorus manure maintaining natural vegetation, increase forage production two to four times, and remove production constraints described. Intensive systems located in more fertile areas are based on cultivated pastures in rotation with cereal or oil crops, where a large group of legumes and grasses can be included in annual, short rotation or perennial pastures (*Trifolium, Medicago, Lotus, Festuca, Dactylis, Lolium, Avena, Triticum, Chichorium).* Forestry and agriculture development is concentrating meat and wool production to marginal areas, reinforcing value of pioneer legume species for low fertile, acid and dry environments, achieving importance the use of genus *Lotus* from general to specialized purposes. As an example of research priorities in the region, the Pastures & Forages program of INIA Uruguay for the 2007-2011 plan emphasis on: a) *sustainable management on natural grasslands* promoting new ways to add value and/or systems that allow biodiversity conservation, b) *forage plant breeding* focusing on adapted and improved forage species, c) *cultivated pasture management* focusing on stress of plant energy, competition, plant nutritional and soil biotic and non-biotic factors affecting productivity and persistence, d) *soil-plant-animal relationships* focuses on better understanding of productive processes and plant dynamics to enhance productivity and sustainability and e) *integrated weeds, insects and diseases management* focuses on the design of technologies and solutions with an acceptable environment impact. Challenges in the "campos" ecosystem will refer to alternatives for marginal environments, better quality and safe products for local and overseas markets and evaluation of the impact of technology on natural resources. Concepts of environment, social and economic sustainability are also reflected in research, integrating pasture and animal knowledge to develop a network for research and technology transfer.

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Seed industry requirements

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Temperate Legumes in Southern America Market Overview:

- Argentina, Chile and Uruguay having good availability of quality seeds. A well established PBR system and government institutions are in general doing a good job.
- Brasil and Chile not having a pool of good genetics and poor quality seeds. PBR on temperate legumes is a problem and government is very bureaucratic.

Alfalfa: More than 5 million hectares and 10 000MT of seed sold every year. More than 350 cultivars, 90% winter active, 20% local research, 80% seed consumption from USA, Australia and Europe. Most important is Argentina and expansion to the north and east.

White Clover: Utilize in most of the pastures mix, with more than 1 million hectares and 1 200 MT of seed sold every year. Around 25 cultivars available but only a couple most important, 20-25% seed imported, 60% labeled seed and 15-20% “white bag” seed. Good seed production from Argentina and Uruguay.

Lotus corniculatus: Utilize in poor, shallow and high acid soils. More than 1,6 million hectares and 5 000 MT of seed sold per year. Around 6 cultivars available but only two are most used. All seed is produced in the region and 80% is done in Uruguay. 20% is labeled seed and 80% is “white bag”. 100% of research and development has been done in the region.

Market Trends: Important forage legume in SA, widely used in different climates and soils. High nutritive value and do not cause bloat. Market appears to be expanding. Exports to Canada, USA and Europe. Propriety cultivars with improved agronomics performance and persistence.

Lotus tenuis: Going into low lands and flooding areas; adapted to low fertility and salinity. More than 200 000 hectares and 500 MT of seed sold per year. 6 cultivars available but 2 are most common. Most used in Argentina and all the seed production done there. 20% labeled seed and 80% “white bag”.

Lotus subbiflorus: Utilize in poor and shallow soils. More than 100 000 hectares and 500 MT of seed sold per year. One cultivar available (from Uruguay). All seed is produced in the region (Uruguay). 20% labeled seed and 80% “white bag”. 100% research done locally.

Lotus pedunculatus: Poorly drained soils in humid areas. More than 20 000 hectares and 50 MT of seed sold per year. Two cultivars are available from overseas research. Seed produced in the region. Mainly used in the northeast of Uruguay. 90% labeled seed.

Demand of new cultivars for *Lotus* spp: Expansion of grain crops over better soils, Greater rainfall variability, Strategic role in long-term pasture mixtures for beef production, Adoption of improved establishment and pasture management technology, Increased use in marginal areas (Semiarid Pampa, Subtropical) where alfalfa does not perform, Mixtures with tall fescue and cocksfoot to improve summer production, Short-lived pasture phase in rotation with soybean and maize for silage, Soil improvement in crop-pasture rotations

General Comments: *Growth of agriculture*. Seed production vs. Commodities. Displacement of pastures to marginal soils. Increase of production costs. Occasional harvest.

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Requirements from the MERCOSUR inoculants industry

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The technology of inoculating crops and legume pastures has been adopted since long time ago in South America. Several efforts have been made in order to develop this natural process into a successfully applied technology. Scientists started working at their laboratories in order to find a *Rhizobium* formulation with the capacity to increase the BNF in plants. In 1886, two German scientists, Hellrigel and Wilfarth, isolated some bacterias from protuberances present in the hairs of the legumes roots. In Argentina, Halbinger is recognised as the first inoculant producer (at the Laboratory of the Ministry of Agriculture of Santa Fe), in 1937. Laboratory products evoluted and in 1956, in Brazil, Dr. Joao Ruy Jardim Freire gave technical assistance in order to install a legume inoculant industry. At the same time, Uruguay was developing experimental inoculants and large scale produced peat based formulations started to be officially controlled in 1963 by Dr. R. A. Date, from The University of Sydney in the "Laboratorio de inoculantes" of the Ministry of Agriculture. In late 80´s, only one Paraguayan legume inoculant industry, "Inoculantes S.R.L.", manufactured a product called "Ybyru", which only was sold for a short period of time. The degree of success of the inoculant companies in each country depend on several factors: commercial, regulatory, technical and technological. Nevertheless, the importance given by each government to the diffusion of the technology and to assure that farmers receive a high quality inoculant is the reason why there is such a difference in inoculated areas in countries of MERCOSUR. It is important to emphasize that in those of them where the inoculation technology is succesfull, with a high level of adoption in commercial legume pastures or crops, there has a permanent and intimate relation between researchers, universities, public entities, farmers and the private sector. Cooperative efforts among all these sectors permitted a dynamic development of the BNF research work, which allowed enormous economical benefits from it in productive terms. Today, soybeans are the legume crop in south America to which most rhizobiological work has been dedicated. In relation to the Lotus genre, the inoculation practise does not have high levels of adoption in all countries, and in most situations the reason is not the lack of positive productive responses. Although we may consider there is great research work dedicated to it in relation to BNF, it has not been transferred to the productive sector (farmers) in the same manner as it occurred with soybeans.