Asociación Española
de Leguminosas
(Spanish Association for Legumes)

PHASEOMICS
VI Meeting

Actas 4

Coordinators:
Antonio M. De Ron, Marta Santalla

Pontevedra (Spain), May 2009

PHASEOMICS
VI Meeting

Actas de la Asociación Española de Leguminosas (Spanish Association for Legumes)

VOL. 4 (2009)

Acción Complementaria AC2008-00047-00-00
financiada por el Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA),
en el marco del Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i)
y por el Fondo Europeo de Desarrollo Regional (FEDER).

Edited by AEL and MBG-CSIC. Pontevedra, Spain. 2009
Coordinators: Antonio M. De Ron and Marta Santalla
Designed and printed by Idea Gráfica. Pontevedra, Spain
Dep. Legal: PO 333-2009

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PRESENTATION

PRESENTATION

This volume (Actas AEL, 4) contains the contributions of scientist from Europe and other countries to the PHASEOMICS VI Meeting, held in Pontevedra, Spain, on May of 2009, organized jointly by the Misión Biológica de Galicia (MBG), CSIC and the Spanish Association for Legumes (AEL).

The scientific programme has been developed by the members of the International Scientific Advisory Board and the organization of the Symposium was in charge of the Organizing Committee.

The first part of the volume includes the introductions to PHASEOMICS, to the CSIC and the MBG, to the AEL and the programme of the Meeting. The second part includes the summaries of the lectures and the posters.

The coordinators of this volume are most grateful to all those who assisted in planning, organizing and developing the Meeting, as well as to all the authors contributing to it and to the PHASEOMICS Chair Prof W. Broughton. In particular some institutions have supported this event as the Ministery of Science and Innovation, INIA, CSIC, Diputación de Pontevedra and Xunta de Galicia, and some other organizations have contributed to make possible this Meeting.

At last the coordinators acknowledge the supporting by the Director of the MBG, and the big and hopeful effort made by the Legumes Group at the MBG, to organize successfully this Meeting in a short period of time, devoting to this aim a lot of time and all their enthusiasm.

Pontevedra, Spain. May, 2009

Antonio M. De Ron
Marta Santalla
Coordinators

INTRODUCTION

More people consume common beans (*Phaseolus vulgaris* L.) than any other grain legume. They are the major source of protein for Americans living south of the Rio Grande to Patagonia. Beans are highly nutritive but unlike meat contain little cholesterol. As a consequence, they are often important components of gourmet meals. Unfortunately, the conclusion that agricultural productivity could simply be increased by extending the area planted to beans stumbles on two major agronomic limitations. Production of dry beans is low even by the standards of legumes, and beans do not fix as much nitrogen from the air as studies with other legumes suggest they could do. Development of designer beans tailored to different markets would also require reduced cooking times, easier digestibility, higher iron (Fe) contents and so on. Similarly, greater resistance to drought, insects, pathogens and other agronomic challenges would lead to steady increases in bean production. Nevertheless, as an object of research, beans have few peers. *P. vulgaris* is a true inbreeding diploid with 11 chromosomes and a haploid DNA complement of ≈ 590 Mb. Its small genome, with little duplication, makes it ideal for genetic studies of all kinds. Furthermore, as the major representative of the agronomically important legume tribe *Phaseoleae* it is representative of such other widely consumed legumes as *Cajanus cajan* (pidgeon pea), *Glycine max* (soybeans), *Pachyrhizus* spp. (yam beans), winged beans (*Psophocarpus tetragonolobus*), as well as *Vigna* spp. (long beans, mung beans). Since soybeans are effectively amphlidiploid, the genome sequence of beans will serve not only as a reference for the entire *Phaseoleae* by will help elucidate more complex genomes like soybeans.

With these challenges in mind, the PHASEOMICS consortium was formed to bring together all people working on beans. By sharing their experiences and common goals, bean research would be accelerated.

In continuing this collegial spirit, Marta Santalla and Antonio M. De Ron agreed to host the sixth Meeting in the series (Phaseomics VI) in Pontevedra, Spain from May 21 to 23, 2009. It is obvious from the programme that Marta and Antonio succeeded not only in bringing together representatives of most major bean groups, but that they have included ample time for the direct inter-personnel interactions that are the real stimulus for research. Our hosts are to be congratulated for their foresight and thanked for their extra-ordinary organisational skills.

William J. Broughton
For the PHASEOMICS Consortium,
Geneva, Switzerland

THE CSIC AND THE MBG

The Spanish Government created in 1907 the **Council for the Extension of Studies and Scientific Research** (“Junta para Ampliación de Estudios e Investigaciones Científicas” - JAE) aiming to end Spain isolation and to enhance links with the European science and culture, being **Santiago Ramón y Cajal**, awarded with a Nobel Prize in 1907, its President. This new body had the mission of training the staff responsible for implementing the reforms needed in science, culture and education. The scientific and cultural programme by the JAE represented the creation of laboratories and research centres and the awarding of fellowships for training study abroad, facilitating that leading Spanish thinkers and scientists contacted with those in different countries. In 1939, the Government created the **Spanish National Research Council** (“Consejo Superior de Investigaciones Científicas”-CSIC) that incorporated all the institutes and laboratories belonging to the later JAE.

Currently, the CSIC is a State Agency that is the Spanish largest and most important public research organization. The mission of the CSIC is to promote, coordinate, develop, and disseminate multidisciplinary scientific and technological research in order to contribute to economic, social, and cultural development and the progress of knowledge. Furthermore, it aims to train research personnel and provide advice to public and private institutions on subjects within its areas of expertise. The CSIC has 126 centers or institutes and 145 associated units, distributed in eight fields of research: Humanities and Social Sciences, Biology and Biomedicine, Natural Resources, Agricultural Sciences, Physics, Chemistry, Materials Science and Technology, and Food Science and Technology.

The Misión Biológica de Galicia (MBG) was founded in 1921 by the JAE, being Cruz Gallástegui its first Director. It was located in the School of Veterinary of Santiago de Compostela until 1926, when the School of Veterinary was located out of the region. In 1927, the Provincial Government (“Diputación Provincial”) of Pontevedra offered the Old Palace of Salcedo as a new location for the MBG. In 1939 the MBG become an institute belonging to the CSIC.

The objective of the MBG was to address the agricultural problems of the main crops of Galicia (maize, potatoes, brassica, chestnut trees) trough a genetical approach. The modern history of the MBG began in 1974 based on maize breeding, collecting firstly a local germplasm collection that was studied from several points of view obtaining a valuable and documented maize germplasm collection that was the base for selection programs to improve adaptation and yield. The MBG grew and new programs were developed based on other crops. In 1987 began the legume (beans and peas) program, in 1990 a collection of grapevines was established, and during these years local varieties of the Brassica crops (kales, turnip greens, turnip tops, and leaf rape kales) were also collected. Collecting, conservation and characterization of genetic resources were the initial priorities in all programs. Currently, there are two research Departments at the MBG, Plant Genetics and Plant Genetic Resources, with research lines focussed on genetics and breeding. The main goal of the MBG is to obtain novel knowledge on the genetic processes involved in adaptation and evolution, resistance to stresses, and quality of crops.

Antonio M. De Ron
Head of Plant Genetic Resources at the MBG

THE NINE YEARS OF THE SPANISH ASSOCIATION FOR LEGUMES ("ASOCIACIÓN ESPAÑOLA DE LEGUMINOSAS" - AEL)

The (AEL) was created as an initiative of a group of Spanish legume researchers whose members were well connected since many years ago. In 1992 was celebrated in Palencia (Spain) the 1st Technical Conference on Grain Legumes that joined 90 persons coming from research institutes, private companies and public administration, all them interested in the Spanish legumes. The Conference conclusions were submitted to the Ministry of Agriculture and was agreed to organize this type of Conference each three years.

Eight years after, during a meeting of the Local Committee of the 3rd Conference on Grain Legumes by the AEP (European Association for Grain Legume), the agreements of the past Technical Conference were revised and was discussed the creation of a Spanish National Association. This idea was presented to the Spanish specialists in a meeting during the Conference and a committee for the promotion of such Association was nominated. The Spanish Association for Legumes ("Asociación Española de Leguminosas" - AEL) was constituted on February of 2000 and was registered by the Home Ministry on May 31. The Spanish Association had been born and it is unquestionable that the 3rd AEP Conference was an appropriate framework for its gestation.

The objectives of the Association are: (1) to promote the Spanish legumes (all the legumes, not only grain legumes), (2) to coordinate the researchers with the agricultural sector, (3) to be a forum for discussion between agricultural sector, researchers and social interlocutors, (4) to preserve legumes variability, and (5) to encourage new legumes uses. In order to develop all these objectives the main AEL activities were:

- To encourage research on legumes in Spain and promote activities to improve sector competitiveness.
- To favour information exchange between persons and organizations both at national and international level
- To promote a high quality productions and encourage consumption.
- To make easy Meetings, Congress and Courses organization and the recompilation and spreading of information.
- To develop the understanding of the benefits of the legumes into the philosophy of a sustainable agriculture.

The running of the Association is on the hands of their members through yearly General Assembly, but the management is delegated in an Executive Committee elected by the members and totally clear out every six years.

The Association has been plenty of activities during these years. A set of basic tools was developed, such as, active members and related contacts databases or tools for publicity as leaflets, technical publications in magazines or advertising through mass media contacts. A web page (www.leguminosas.es) displays the principal events of the Association.

Every tree years a National Conference is organized. The first one was hold in Córdoba from the 15 to the 17 December 2003, the second one in Cuenca from the 25 to 27 April and the last one in Valladolid from the 2 to the 4 September 2008. In all these Conferences a very full, wide-ranging program was presented. The main lectures showed an excellent scene of legumes in Spain, regarding to the past, the present and the future of legume crops in our country. A large number of communications, both oral and poster, covered more conventional topics as: the role of legumes in crop rotations; the use of legumes in human and animal diets and their effects in human health; legumes non nutritive factors and their possible industrial utilization; the effect of legumes seed extrusion on nutritional quality; *Rhizobium* strain selection; the use of molecular markers in plant breeding; pollination and legumes flowers; genetic diversity and its conservation, characterization and evaluation; diseases, resistance and control; etc. All the papers from these Conferences were recovered in the appropriate Proceedings.

Since AEL was constituted, the cooperation with other National Associations related was established. The link between AEL and the Researchers Group for the study of Iberian *Phaseolus vulgaris* has been especially fruitful. In fact, the first and second AEL publications are the Proceedings of the Second and Third Seminars of Iberian Peninsula Bean. As a natural consequence of this collaboration, the Third AEL Conference and the Fourth Seminar of Iberian Peninsula Bean, were developed at the same time in Valladolid.

AEL have participated in the majority of the Spanish initiatives related with legumes. So, their members have collaborated with Ministries of Science and Innovation Agriculture, Food and Fisheries, Environment; the Nutrition and Health National Network, the National Seed Producers and some Farmers and Rural Development Associations. AEL is also one of the Spanish Scientific Associations founder members of the COSCE (Confederation of Scientific Societies of Spain). The objectives of such Confederation are: (a) scientific and technologic development in Spain, (b) to be the official negotiator with civil society and public representatives and (c) to cooperate in the development of a social scientific culture in our country.

The AEL has also participated in many International Projects mainly involved in the dissemination work packages. Its role in some of them was:

- Last Meeting for the dissemination of the results of the project PHASELIEU "Improvement of sustainable *Phaseolus* production in Europe for human consumption". This project was a Concerted Action supported by the specific programme FAIR (Fisheries and agriculture) of the European Commission from January 1998 till March 2001
- Dissemination Event (Madrid, April 2002) organized by LINK, a Legumes Interactive Network supported by the programme FAIR of the European Commission from January 1999 to September 2002, and their accompanying measure "Pulses and Health" supported by the key action "Quality of Life".
- Canada Pulses Day 2005, cooperation with the Canadian Legumes Association to present Spanish legumes in this fair hold in Saskatoon, Saskatchewan, January 10 – 11.
- Participation of AEL as a member of the consortium of the European Project GLIP, Grain Legumes. New strategies to improve grain legumes for food an feed belong to the Sixth Framework Programme, Priority 5: Food Quality and Safety (February 2004 – February 2008),being the organization of two Dissemination Events, Madrid, October 2005 and Valladolid, October 2007, its principal objective.

At the present, is a great pleasure for our Association the cooperation with the PHASEOMICS Global Initiative Work Group in the organization of the PHASEOMICS VI Meeting (Pontevedra, 2009 May 21 – 23). All the members of AEL welcome the participating researchers in this Meeting and wish that PHASEOMIC VI had a happy outcome.

Dr Celia de la Cuadra
First AEL President 2000-2006

PHASEOMICS VI MEETING
PONTEVEDRA, SPAIN. 21-23 MAY 2009

PROGRAMME

ORGANIZATION

The Misión Biológica de Galicia (MBG) of the Spanish National Research Council (CSIC) and the Spanish Association for Legumes (AEL) organize this Meeting in the old Palace of Salcedo, in the MBG, Pontevedra, Spain.

COORDINATORS

Prof Antonio M. De Ron & Dr Marta Santalla.
Misión Biológica de Galicia, CSIC. Pontevedra, Spain
Spanish Association for Legumes. Alcalá de Henares, Spain

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Dr Ana M. González

MSc Manuel Riveiro

MSc Soledad Saburido

SCHEDULE

Thursday, May 21				
13.45	Departure to the MBG		Bus (see maps 1 and 2)	
14.00 – 16.00	Registration (Authors: to fix the posters) (Authors: to deliver the presentation files)		Palace Registration Desk	
16.00 – 16.30	Opening Ceremony		Palace Hall	
16.30 – 17.30	Opening session		Palace Hall	
17.45	Departure to Pontevedra	*Satellite workshop: group for common bean genome sequencing (CyTED Proposal)*	Bus	Palace Meeting room
18.00 – 19.00	Walk along the old town		Pontevedra downtown	
19.30 – 20.30	Welcome Party		Council for the Denomination of Origin of "Rías Baixas" wine. Mugártegui Palace Plaza da Pedreira 10 (see map 3)	
Friday, May 22				
8.45	Departure to the MBG		Bus (see map 1)	
09.00 – 11.00	Session 1		Palace Hall	
11.00 – 11.30	Coffee break		Gardens / Palace	
11.30 – 13.00	Session 1		Palace Hall	
13.30 – 15.30	Lunch		Batacos Lodge, beside the MBG	
15.30 – 17.00	Visit to the MBG	*Satellite workshop: group for common bean genome sequencing (CyTED Proposal)*	Palace, research buildings, gardens and facilities	Palace Meeting room
17.00 – 18.00	Poster session and drinks		Palace	
18.15	Departure to Pontevedra		Bus	
20.00	Departure to Soutomaior		Bus	
20.30 – 23.00	PHASEOMICS Dinner		Soutomaior Castle, 13 km away from Pontevedra (see map 4)	
23.15	Departure to Pontevedra		Bus	
Saturday, May 23				
08.45	Departure to the MBG		Bus (see map 1)	
09.00 – 11.00	Session 2		Palace Hall	
11.00 – 11.30	Coffee break		Gardens / Palace	
11.30 – 13.30	Session 3		Palace Hall	
13.30 – 15.30	Lunch		Gardens / Palace	
15.30 – 17.30	Session 4		Palace Hall	
17.30 – 18.30	Drinks and farewell (Authors: to remove the posters)		Gardens / Palace	
18.45	Departure to Pontevedra		Bus	

Opening Ceremony:

Welcome by representatives of institutions

Opening session. Chair: Antonio M. De Ron. MBG-CSIC. Spain

Welcome to PHASEOMICS VI

William Broughton.
University of Geneva. Switzerland

Marta Santalla.
MBG-CSIC. Spain

Antonio M. De Ron.
MBG-CSIC. Spain

Opening lecture:

Georgina Hernández
CCG. México
BEAN GENOMICS: FUNCTIONAL GENOMICS OF ABIOTIC STRESS RESPONSE

Session 1. Chair: Marcelino Pérez de la Vega. University of León. Spain

Genomics of *Phaseolus vulgaris*

Simon Santa Cruz, Laureano Simón
Proteomika. Parque Tecnológico de Zamudio. Derio, Vizcaya. Spain
THE APPLICATION OF PROTEOMICS TO PLANT SCIENCE - LIMITATIONS AND SOLUTION

Tim Porch , N.M. Boukli And William J. Broughton.
USDA/ARS/TARS, Mayaguez, Puerto Rico. Universidad Central del Caribe, School of Medicine, Department of Microbiology and Immunology, Bayamon, Puerto Rico. LBMPS, Université de Genève, Sciences III. Genève, Switzerland.
TILLING AND PROTEOMICS IN Phaseolus vulgaris *cv BAT93.*

Federico Sánchez, Juan Elías Olivares, Georgina Estrada-Navarrete, Margarita Rodríguez-Kessler, Xóchitl Alvarado-Affantranger, Claudia Diaz-Camino, Gabriel Guillen, Guadalupe Zavala, Carmen Quinto, Ildefonso Bonilla.
Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México. Universidad Autónoma de Madrid, Madrid, Spain
IS nod30 PART OF AN ANTI-CELL DEATH PROGRAM IN Phaseolus vulgaris *ROOT NODULES?*

Panzeri D.; Tagliabue G.; Martinelli T.; Ramirez M.; Valdes Lopez O.; Hernández, G.; Sparvoli F.
Institute of Biology and Agrarian Biotechnology, CNR, Milan, Italy. Centro de Ciencias Genómicas, UNAM, Cuernavaca, Morelos, México
FUNCTIONAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE BEAN lpa-280-10 MUTANT

Tereza Cristina de Oliveira Borba; Flávio Breseghello; Maria José Del Peloso; Leonardo Cunha Melo; Helton Santos Pereira; Rosana Pereira Vianello Brondani
EMBRAPA, Brasil
MOLECULAR MARKERS AS TOOLS FOR THE SELECTION OF GERMPLASM ACCESSIONS FOR THE EMBRAPA'S COMMON BEAN'S CORE COLLECTION

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Depto. De Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México.

SIGNALING AT THE EARLY STAGES OF THE BEAN-RHIZOBIA SYMBIOSIS: APPROACHES AND ACHIEVEMENTS

O. Mario Aguilar, María E. Zanetti, María P. Beker, Eitel Peltzer Meschini and Flavio A. Blanco

Instituto de Biotecnología y Biología Molecular, Facultad de Ciencias Exactas, UNLP, CCT Conicet La Plata, Argentina

EARLY RESPONSES OF Phaseolus vulgaris TO SPECIFIC STRAINS OF Rhizobium etli: ROLES OF A MONOMERIC G PROTEIN AND A CCAAT TRANSCRIPTION FACTOR

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INRA-IRD-SUPAGRO, UMR1222 Eco&Sols (Ecologie Fonctionnelle & Biogéochimie des Sols), Montpellier, France

INTERACTIONS BETWEEN N_2 FIXATION AND PHOSPHOROUS BIOGEOCHEMICAL CYCLE FOR LEGUME CONTRIBUTION TO SUSTAINABILITY IN AGRICULTURE

Session 3. Chair: Francesca Sparvoli. CNR. Italy

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Marsolais, F.; Pajak, A.; Fuqiang, Y., ; Shangzhi, H.; Chapman, R.

Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Canada. Department of Bioscience & Biotechnology, School of Life Sciences, Sun Yat-sen University, Guangzhou, China

PROTEOMIC ANALYSIS OF SEED STORAGE PROTEIN DEFICIENCY IN COMMON BEAN

Wright, L.; Smith, T.; Pauls, K.P

Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada N1G 2W1

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CARB center, Department of Molecular Biology, University of Aarhus, Denmark. iThemba LABS, Somerset West, South Africa. Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Cracow, Poland. Dept. of Pediatrics, Baylor College of Medicine. USDA-ARS Children's Nutrition Research Center, Houston, TX, USA. Dept. of Agricultural Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark

ARE Lotus SPECIES GOOD MODELS FOR STUDYING IRON ACCUMULATION IN COMMON BEANS?

Domenico Perrone, Bruno D'Onofrio, Bruno Campion
CRA-Centro di Ricerca per l'Orticoltura, Battipaglia, Italy. CRA-Unità di Ricerca per l'Orticoltura, Montanaso Lombardo, Italy
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Session 4. Chair: William Broughton. University of Geneva. Switzerland

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Quo Vadis PHASEOMICS? – general discussion

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Group for common bean genome sequencing (CyTED Proposal)

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POSTERS

Number	Authors	Affiliation	Title
1	Bernal, C.; Gamero Moises; Galindo, Ivan; Diez, Nardy.	Centro de Biotecnología, Laboratorio de Genómica y Proteómica, Instituto. Fundación Instituto de estudios Avanzados IDEA. Caracas, Venezuela.	PROTEOMIC CHARACTERIZATION OF ARCELIN AND PHYTOHEMAGGLUTININ SEED PROTEINS FROM *Phaseolus vulgaris*
2	M. De la Fuente, G. Álvarez, C. Zapata, A. Borrajo, M. Santalla	Misión Biológica de Galicia, CSIC. Pontevedra. Spain. Department of Genetics. University of Santiago de Compostela, Spain.	FIRST APPROXIMATION TO THE STUDY OF THE PROTEOME OF MESOAMERICAN AND ANDEAN BEAN GENOTYPES
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4	M. Riveiro, A. M. De Ron, J. J. Drevon, A. P. Rodiño	Misión Biológica de Galicia, CSIC. Pontevedra. Spain. INRA. Montpellier. France	INTERACTION OF WATER STRESS AND NODULATION IN BEAN LANDRACES.
5	Estévez, J.[1], Manyani, H.[1], Soria-Diaz, M.E.[2], J., Gil-Serrano, A.[2], Van Brussel, A.A.N.[3], Megias, M[1].	[1.] Dpto de Microbiología y Parasitología, Facultad de Farmacia. [2.] Dpto. De Química Orgánica, Facultad de Química. Universidad de Sevilla. España. [3.] Institute of Biology Leiden, Clusius Laboratory, Leiden University. The Netherlands.	*Phaseolus vulgaris* ROOT RESPONSES TO *Rhizobium tropici* CIAT899 Nod FACTORS PRODUCED UNDER ABIOTIC
6	Roser Romero del Castillo[1], Antoni Almirall1, Elena Pérez-Vega[2], Juan José Ferreira[2], Francesc Casañas1	[1]Departament d'Enginyeria Agroalimentària I Biotecnologia. UPC. ESAB. Campus del Baix Llobregat. Castelldefels, Spain. [2]SERIDA. Villaviciosa. Asturias, Spain.	*SENSORY EVALUATION OF NEAR ISOGENIC LINES DERIVED FROM THE FABADA MARKET CLASS A25 LINE*
7	Urbanski D. F.[1], Sørensen K.[1], Jurkiewicz A.[1], Przybyłowicz W. J.[2,3], Mesjasz-Przybyłowicz J.[2], Stougaard J.[1], Jensen E. Ø.[1], Cvitanich C.[1]	[1] CARB center, Department of Molecular Biology, University of Aarhus, Denmark [2] iThemba LABS, Somerset West, South Africa [3] Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Cracow, Poland.	*IRON, ZINC, AND FERRITIN ACCUMULATION IN COMMON BEANS*
8	Teresa Hernández[1], Soledad Diaz1, Yolanda Aguilera[2], Isabel Estrella[1], María A. Martín-Cabrejas[2], Rosa M. Esteban[2]	[1]Instituto de Fermentaciones Industriales. CSIC. Madrid. Spain. [2]Departamento de Química Agrícola. Facultad de Ciencias. Universidad Autónoma de Madrid (UAM). Madrid. Spain	*DIFFERENCES IN THE PHENOLIC COMPOSITION OF SOME Phaseolus VARIETIES*
9	Lioi, L.[1]; Campion, B.[2]; Piergiovanni, A.R.[1]	[1] Istituto di Genetica Vegetale-CNR, Bari, Italy [2] CRA-Unità di Ricerca per l'Orticoltura, Montanaso Lombardo, Lodi, Italy	*GENETIC DIVERSITY AND TECHNOLOGICAL TRAITS OF A COMMON BEAN LANDRACE FROM SICILY (ITALY): FAGIOLO A BADDA*

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11	J. S. Bayuelo-Jiménez[1], I. Ochoa[2]	[1]Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Michoacán, México. [2]Corporación Colombiana de Investigación Agropecuaria CORPOICA, Villavicencio, Colombia.	CARBOHYDRATES AND INORGANIC ION CHANGES DURING SALT STRESS EXPOSURE OF *Phaseolus* SPECIES: INVOLVEMENT ON GROWTH, WATER RELATIONS AND OSMOTIC ADJUSTMENT
12	Trifan, D.[1]; Leonte, C.[2]	[1]"Low Danube" University from Galati, Romania, Faculty of Mechanical Engineering from Braila, Department of Agriculture and Ecology. [2] UŞAMV Iaşi, Faculty of Agriculture	RESEARCH CONCERNING THE PHENOTYPIC VARIABILITY OF SOME GARDEN BEAN HYBRIDS (*Phaseolus vulgaris L.*)
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14	M. Santalla, A. M. González, M. Lores, A. Castro, A. M. De Ron, M. De la Fuente	Misión Biológica de Galicia, CSIC. Pontevedra. Spain	CHARACTERIZATION OF RESISTANCE LOCI TO THE ANTHRACNOSE PATHOGEN (*Colletotricum lindemuthianum L.*) IN ANDEAN AND MESOAMERICAN BEANS.

WEBSITE
www.phaseomicsvi.net

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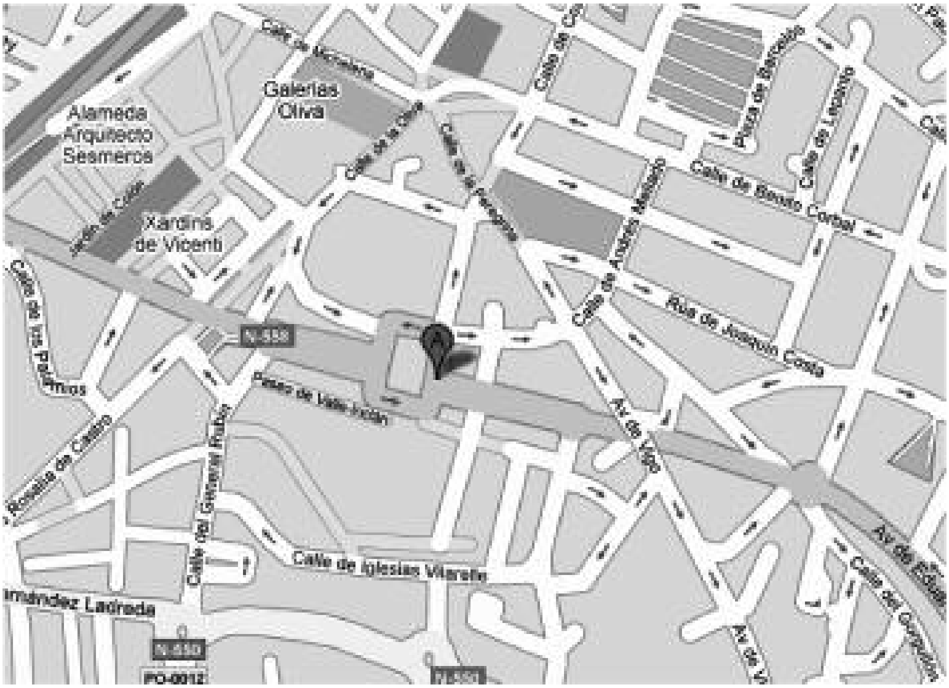
Ministry of Science and Innovation: www.micinn.es
Misión Biológica de Galicia (MBG – CSIC): www.mbg.csic.es
Spanish National Research Council (CSIC): www.csic.es
National Institute for Agricultural Research (INIA): www.inia.es
Galician Directorate for R&D&I (IN.CI.TE): www.conselleriaiei.org/ga/dxidi/index.php
Provincial Government (Diputación de Pontevedra): www.depontevedra.es

CO-OPERATORS
Council for the Denomination of Origin "Rías Baixas" wine
Council for the Denomination of Origin "Ribeira Sacra" wine
Council for the Denomination of Origin "Faba de Lourenzá" dry bean
Quival. S.A. - Frutos secos El Nogal

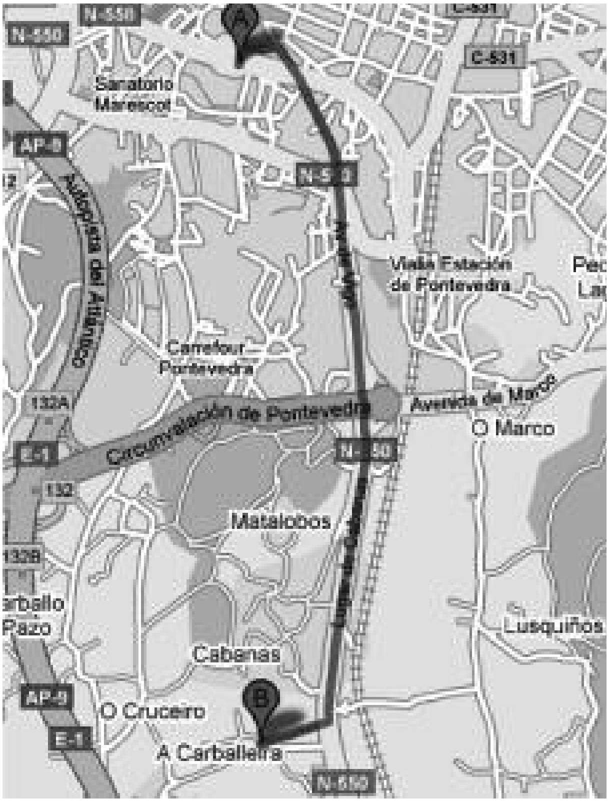
MEETING TRANSPORTATION:

The organization will provide transportation from Pontevedra downtown to the venue of the Meeting (Palace of Salcedo-MBG) and return, and to Soutomaior Castle for the PHASEOMICS Dinner and return.



Map 1

A: Location (Plaza de Galicia) of the stop of the bus going (and returning) to the Palace of Salcedo-MBG and to Soutomaior Castle. Departures are scheduled 15 min before the beginning of each session and 15 min after the end



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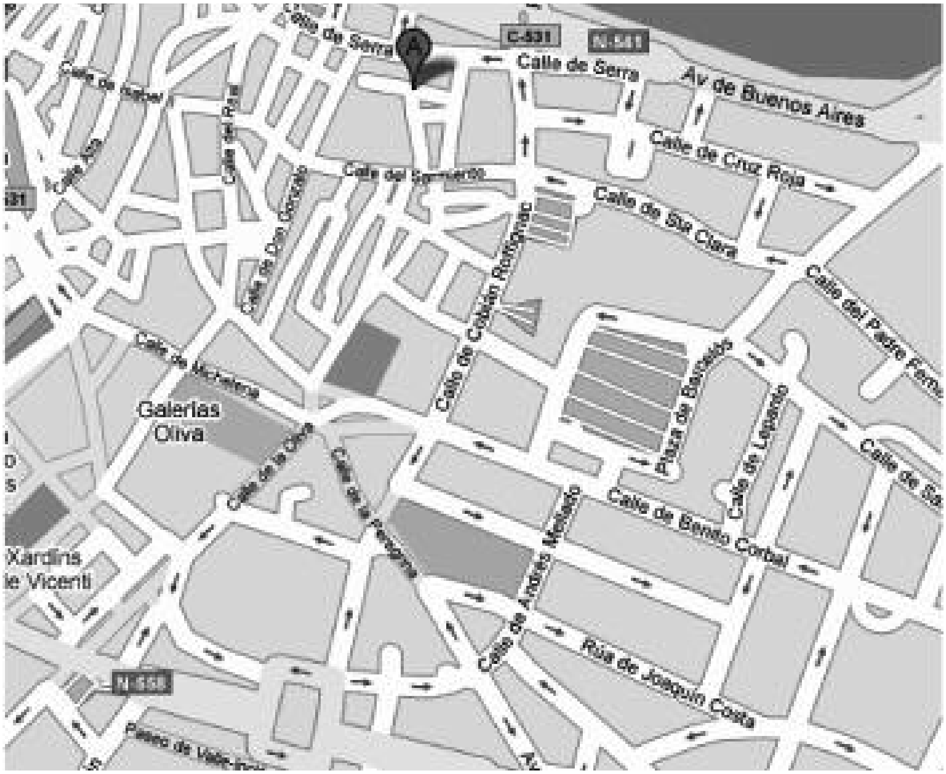
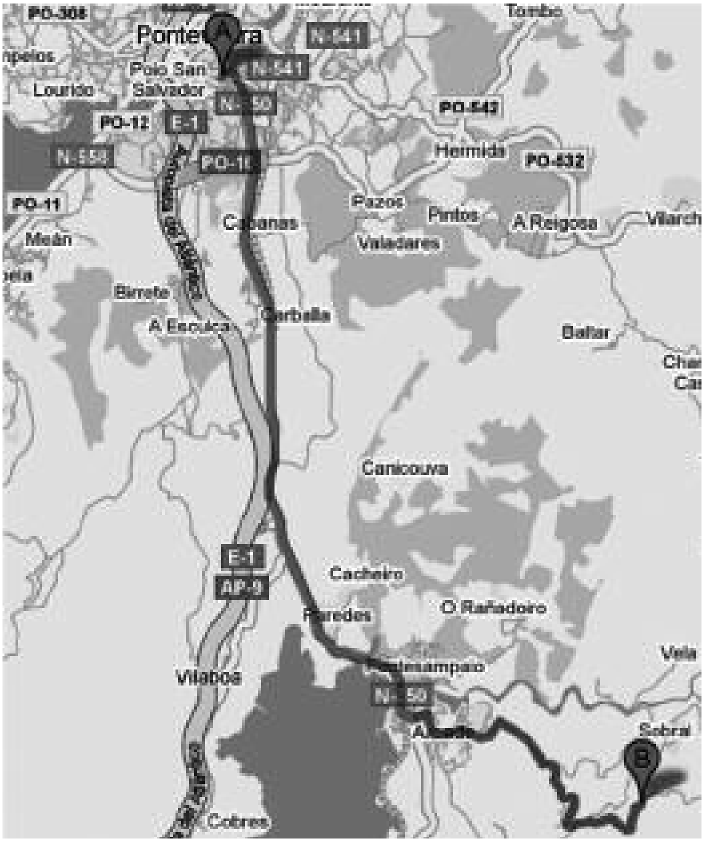
Map 2

Itinerary from A-Pontevedra downtown to B-Palace of Salcedo-MBG



Map 3

A: Council for the Denomination of Origin "Rías Baixas" wine.
Mugártegui Palace. Plaza da Pedreira 10



Map 4

Itinerary from A-Pontevedra downtown to B-Soutomaior Castle

OPENING LECTURE

FUNCTIONAL GENOMICS OF COMMON BEAN RESPONSES TO ABIOTIC STRESS

Hernández, G.[1]; Valdés-López, O.[1]; Ramírez, M.[1]; Girard, L.[1]; Sánchez, F.[2]; Reyes, J. L.[2]; Graham, H. P.[3]; Vance C.P.

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[3] University of Minnesota, St. Paul, MN, USA.

In Latin America and Africa, the yield of common bean production is low, in part because disease and insect pressure but also because of edaphic constraints that include soil nutrient (nitrogen and phosphorus) deficiency, soil acidity and metal toxicity. Current research from our groups aims to decipher the response of bean plants to abiotic stress, using our platform for functional genomics research that includes: transcriptomics (macroarrays and soybean Affymetrix gene chips), metabolomics, transcription factors and microRNAs expression profile and reverse genetics. We have concentrated in analyzing the response to: a) phosphorus deficiency, b) manganese toxicity and c) oxidative stress after Paraquat exposure, of bean plants in non-symbiotic conditions as well as plants in symbiosis with rhizobia. Besides global transcriptome analysis we have analyzed differential gene expression of key regulators such as transcription factors (TF) (qRT-PCR platform) and microRNAs (miRNAs). The approach used to define bean organ-responsive miRNAS included the design of mini-arrays, spotted with synthesized 21-mers from conserved Arabidopsis and novel soybean and common bean miRNAs (Arenas et al. submitted) which were hybridized to radio-labeled miRNA-enriched RNA samples obtained from organs of bean plants grown under control vs. stress treatments. We found that 18 miRNAs showed changes in accumulation under the different nutritional conditions tested. Differential expression of selected miRNAs in stressed bean tissues was confirmed by Northern blot analysis.

Our studies have opened new possibilities to elucidate the sensing, signaling and regulation of the abiotic stress responses in bean plants. The reverse genetic approach: RNAi gene silencing over-expression in composite plants with transgenic roots is being used to decipher the role/relevance of selected bean TF and miRNAs in signaling pathways. We have demonstrated that the MYB family TF: PvPHR1 is a positive regulator of genes implicated in P transport, remobilization and homeostasis in P deficient bean roots. In addition we demonstrated that PvmiR399 is an essential component of the PvPHR1 signaling pathway.

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Valdés-López O, C. Arenas-Huertero, M. Ramírez et al. 2008. Essential role of MYB transcription factor: PvPHR1 and microRNA: PvmiR399 in the phosphorus deficiency signaling in common bean roots. Plant Cell Environ. 31: 1834-1843

SESSION 1

THE APPLICATION OF PROTEOMICS TO PLANT SCIENCE - LIMITATIONS AND SOLUTIONS

Simon Santa Cruz, Laureano Simón

Proteomika. Parque Tecnológico de Zamudio. Derio, Vizcaya. Spain

Proteomics, one of the plethora of "omics" technologies that came to prominence at the turn of the millennium, has been with us now for over a decade but was relatively slow to take hold as a routine tool in plant science research. However, both comprehensive proteomic studies and differential proteomics have been increasingly applied in plant biology over the last few years, triggered in part by the completion of the *Arabidopsis thaliana* genome sequence and subsequently by the sequencing of a wide range of other important crop species.

Although the proteomic analyses of other crops and model systems are increasingly well established there are complexities encountered in working with plants that often go beyond those found when working with other organisms. Key issues include the rigidity of the plant cell wall and the relatively low level of protein per unit of wet weight of tissue in most vegetative plant tissues. These factors combine to make both the extraction and concentration of protein samples problematic. Additionally the wide variety of complex secondary metabolites, sometimes at high levels, encountered in many plant tissues can significantly degrade the quality of the final sample. For example, tannins and other polyphenolic compounds are abundant in many plant tissues, will readily complex with proteins, and can seriously affect sample quality. While the abovementioned factors have complicated the proteomic analysis of plants the careful application of sample extraction and preparation methods that have been developed in many laboratories over the last decade have gone a considerable way to ironing out sample variability due to the preparative procedures employed.

In contrast there is a further cause of sample variation both within a given experimental setting and between experiments that has received little attention because until recently it has been impossible to control. The variation in question arises as a direct consequence of the extreme sensitivity of plants to their growing environment with regard to: light intensity, light quality, photoperiod, temperature, humidity and carbon dioxide concentration, among others. Controlling for the above variables in large scale experiments has been virtually impossible to date but in our opinion the variation caused by uncontrollable variables in glasshouse and even growth chamber experiments is a significant contributor to artefacts in plant proteomic studies. However, recent technological advances are allowing highly controlled experimental settings to be reproduced 365 days of the year using innovative technological solutions. The contribution of this latest generation of controlled environment buildings to the improvement and standardisation of plant proteomic studies will be discussed.

TILLING AND PROTEOMICS IN *Phaseolus vulgaris* CV BAT93.

T. Porch[1], N.M. Boukli[2] and W.J. Broughton[3].

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[3] LBMPS, Université de Genève, Sciences III, Genève, Switzerland.

We evaluated the use of ethyl methane sulfonate (EMS) for the generation of a mutant population for TILLING in common bean. Based on overall survival, development, and yield of treated seed, 40 mM EMS was found to be an appropriate concentration. Higher concentrations of EMS resulted in survival rates of less than 10% and lower concentrations resulted in the generation of fewer mutants. Based on TILLING results from other species, a population of 5000 lines is estimated to be sufficient for saturation of the common bean genome. Phenotypic mutation frequencies and the isolation of targeted mutations in the BAT 93 mutant population indicate that mutagenesis was effective. Proteins were extracted from seeds of four development stages, subjected to two-dimensional-polyacrylamide gel electrophoresis (2D-PAGE) and the spots subjected to MALDI-TOF spectrophotometry. The latest data on both projects will be presented.

IS NOD30 PART OF AN ANTI-CELL DEATH PROGRAM IN *Phaseolus vulgaris* ROOT NODULES?

Federico Sánchez[1], Juan Elías Olivares[1], Georgina Estrada-Navarrete[1], Margarita Rodríguez-Kessler[1], Xóchitl Alvarado-AFFANTRANGER[1], Claudia Díaz-Camino[1], Gabriel Guillén[1], Guadalupe Zavala[1], Carmen Quinto[1], Ildefonso Bonilla[2].

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The symbiotic interaction between legumes and rhizobia is a highly specific and coordinated process that culminates with the formation of a nitrogen-fixing root nodule. Recognition of both symbionts depends on the exchange of molecular signals such as the Nod factors. Rhizobia attach to root hair cells and after recognition, plant cell wall degradation and the subsequent penetration of root hair cells by means of an infection thread occurs. Concomitantly, in the root cortex a new meristem is induced. Bacteria are released from the infection threads into some of the meristem replicating cells by endocytosis, and become surrounded by a plant-derived membrane, the peribacteroid membrane (PBM), which creates the frontier between rhizobia and the cell's cytoplasm. Bacteria inside the PBM multiply, enlarge, and finally differentiate into their specialized N_2-fixing form, the bacteroid. This process gives rise to the infected cells which may contain more than 20 000 bacteroids per cell, interestingly these cells seem to be surrounded by uninfected cells in the nodule's central zone. What are the cell factors that allow infected cells to survive until nodule senescence? We would like to propose that several plant pro-survival signals are active during the symbiotic interaction. In this regard, we will present evidence that members of the Nod30 gene family are nodule specific proteins required to block plant cell death in the infected and un-infected cells in bean nodules.

This work was partially supported by grants IN214909 and CONACYT 83324.

FUNCTIONAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE BEAN LPA-280-10 MUTANT

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[2] Centro de Ciencias Genómicas, UNAM, Cuernavaca, Morelos. México

Phytic acid ($InsP_6$) is the main responsible for poor micronutrient bioavailability to humans, in fact it forms mixed salts with various mineral cations, e.g. calcium, iron and zinc that, when bound to it, are poorly absorbed in the intestine and are largely excreted, resulting in micronutrient deficiencies. In common bean, $InsP_6$ content is a serious problem for human nutrition, particularly in developing countries. By screening an EMS mutagenised bean population we have identified the *lpa* (low phytic acid) 280-10 line that carry a monogenic recessive mutation conferring a 90% reduction of phytic acid content and a 30% reduction of raffinosaccharides to the seed (Campion et al., 2009).

Differently from *lpa* mutants in other species, the bean *lpa*-280-10 apparently does not display negative pleiotropic effects associated to the mutation, is not mutated in any of the known structural genes involved in phytic acid synthesis, and, very important, it shows a better tolerance to drought compared to the wild type. In order to understand the nature of this mutation we have undertaken a transcriptional analysis during seed development of the *lpa* mutant using macroarrays representative of 2,951 unique genes expressed during bean pod development (Ramírez et al. 2005). Preliminary results indicate that at very early stages of *lpa*-280-10 seed development many genes are down-regulated while only few are slightly induced. The transcriptional analysis suggests that this mutant might be in a sort of constitutive drought-stress situation. In fact, Degenkolbe et al (2009) in a transcriptional analysis to study drought tolerance in rice reported the down regulation of genes involved in photosynthetic light reactions, in particular those of photosystem II, in isoprenoid metabolism and a number of protein synthesis genes, especially involved in amino acid activation and synthesis of ribosomal proteins. We have also found many down regulated genes belonging to these categories. Many other down regulated genes fall in the "signalling" and "protein modification" categories suggesting some impairments in signal transductions in the *lpa*-280-10 mutant.

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MOLECULAR MARKERS AS TOOLS FOR THE SELECTION OF GERMPLASM ACCESSIONS FOR THE EMBRAPA'S COMMON BEAN'S CORE COLLECTION

Tereza Cristina de Oliveira Borba; Flávio Breseghello; Maria José Del Peloso; Leonardo Cunha Melo; Helton Santos Pereira; Rosana Pereira Vianello Brondani

Embrapa Arroz e Feijão. Santo Antonio de Goiás. Brazil

A germplasm core collection must represent, with a minimum number of accessions, great fraction of the genetic diversity stored in the originals collections. The selection of accessions must reduce the possibility of replicas. The microsatellite markers are very important tools in the characterization of genetic resources due to their high information content. This work allowed the molecular characterization of two sets of common bean germplasm from the Embrapa's Gene Bank: the first one was a group of Brazilian landraces and the second one was from foreign germplasm. A total of 211 accessions were evaluated by 23 microsatellite markers. This approach, despite the usual utilization in genetic diversity analysis, allowed the identification of potential germplasm accessions for the composition of the common bean's core collection. The molecular data allowed the identification of the most divergent accessions as well as the accessions with very close genetic profile. Such procedure makes the option for direct integration of the most divergent accessions easier, additionally, it also permitted the suggestion for complementary analysis with the accessions that were genetically close, for their elimination or not.

SESSION 2

SIGNALING AT THE EARLY STAGES OF THE BEAN-RHIZOBIA SYMBIOSIS: APPROACHES AND ACHIEVEMENTS

Quinto, C,; Cárdenas, L.; Sánchez-López, R.; Nava, N.; García, K.; Jáuregui, D., Montiel, J.; Santana, O.; Guillén, G.; Mendoza, A.; Sánchez, F.; Alvarado, X.

Depto. de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México., Cuernavaca, Morelos, México.

Establishment of symbiosis between legume plants and nitrogen-fixing bacteria starts when rhizobia recognize flavonoid compounds excreted by the plant root and, in turn, synthesizes the nodulation factors (NF). Perception of NF by the host root hair cells induces ion changes, cytoplasmic alkalinization, calcium oscillations, membrane depolarization, actin rearrangements, and gene expression, among others, that lead to bacterial invasion and nodule formation (Cárdenas et al., 2000). In an attempt to unravel the molecular mechanisms associated to the early events in legume symbiosis, we have initiated a long term project based on a functional genomics study using *Phaseolus vulgaris* and *Rhizobium etli* symbiosis as a model. To do this, two cDNA libraries were constructed either from RNA expressed in roots of Mesoamerican cultivar Negro Jamapa inoculated with *R. etli* (1-72 h *post*-inoculation) or using a suppressive substractive hybridization (SSH) protocol to select sequences expressed as an early response to NF (1-12 h). From the first library, a full-length cDNA coding for a receptor-like kinase with Leu-Rich Repeats domains (LRR-RLK) was isolated and its role at the initial stages of the bean–rhizobia interaction is under characterization. Analysis of knock-down transgenic *P. vulgaris* roots expressing different down-regulated *Pv*LRR-RLK levels suggests that this kinase plays an essential role in nodule formation.

Furthermore, we are interested in studying the earliest cellular responses in living root hair cells to NF using specific fluorescent probes. Recently, a fast a transient intracellular ROS changes responding to NF was found, which appear to be characteristic of the symbiotic interaction (Cárdenas et al, 2008). These results will be presented and discussed.

Partially supported by DGAPA, UNAM IN204907 (CQ) and CONACyT56531.

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EARLY RESPONSES OF *Phaseolus vulgaris* TO SPECIFIC STRAINS OF *Rhizobium etli*: ROLES OF A MONOMERIC G PROTEIN AND A CCAAT TRANSCRIPTION FACTOR

O. Mario Aguilar, María E. Zanetti, María P. Beker, Eitel Peltzer Meschini and Flavio A. Blanco

Instituto de Biotecnología y Biología Molecular, Facultad de Ciencias Exactas, UNLP, CCT Conicet. La Plata, Argentina.

We have previously reported the identification of genes differentially expressed in the strain specific response of *P. vulgaris* to its symbiotic partner *Rhizobium etli* . Transcripts from a group of these genes accumulate after infection of a Mesoamerican bean roots with a strain from the same geographic region, but not when roots were infected with a strain from the Andean region. Subtle or no differences were observed when an Andean bean cultivar was inoculated with its cognate strain, suggesting involvement of these genes in the cultivar-strain specific response (Peltzer Meschini et al, 2008). In order to assess the functionality of these genes in symbiosis, composite plants with increased (overexpression) or reduced (RNAi strategy) levels were generated by *Agrobacterium rhizogenes* mediated transformation.

Silencing of a monomeric G protein belonging to the Rab family produced a reduction in the number and length of the root hairs compared with control GUS RNAi plants. This gene is preferentially accumulated in root hairs and its transcript levels are increased after infection with a cognate strain of *R. etli*, with a maximum at 12 hs after inoculation. Nodulation was completely abolished in Rab RNAi plants, whereas overexpression did not have any noticeable effect on the phenotype. Bacterial attachment to the root was similar between Rab RNAi and control plants, but neither root hair curling nor infection thread formation was observed in roots were Rab transcrips were reduced. Furthermore, accumulation of early nodulins after rhizobial infection was severely compromised in Rab RNAi plants, suggesting this Rab protein to play a role in the early response of the root hair that leads to infection thread formation and induction of nodulin genes.

PvHAP5 encodes a protein highly homologous to the C subunit of CCAAT heterotrimeric transcription factors. PvHAP5 mRNA were found to accumulate at higher levels in *P. vulgaris* roots of the Mesoamerican cultivar at early time points, as well as in young and mature nodules, after inoculation with a *R. etli* strain from the same region than in roots inoculated with a strain from the Andean region. RNAi mediated silencing of PvHAP5 dramatically reduced the number of nodules respect to control plants (GUS-RNAi) giving a similar number of nodules when inoculated with either the Mesoamerican or the Andean strain. On the other hand, in PvHAP5 overexpressing hairy roots, inoculation with a rhizobial strains from Mesoamerica did not significantly changed nodulation efficiency. By contrast, inoculation with Andean strains resulted in formation of higher number of nodules in PvHAP5 overexpresing than in control roots (about 2 folds), reaching values that were comparable to the number of nodules per root developed upon inoculation with the Mesoamerican strains. These results suggest that PvHAP5 might mediate cultivar-strain specificity during the symbiotic association between *P. vulgaris* and *R. etli*

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INTERACTIONS BETWEEN N_2 FIXATION AND PHOSPHOROUS BIOGEOCHEMICAL CYCLE FOR LEGUME CONTRIBUTION TO SUSTAINABILITY IN AGRICULTURE

Jean-Jacques Drevon, Nora Alkama, Laurie Amenc, Adelson Apaujo, Benoit Jaillard, Saber Kouas, Fatma Tajini, Mainassera Zaman-Allah.

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Legumes have the capacity to fix large amounts of atmospheric N_2 into the biosphere through their capacity to establish a symbiosis with soil rhizobia. However this legume contribution to the N biogeochemical cycle varies with the nodulated-root rhizospheric environment, and P availability in particular. In order to assess the environmental constrains that might limit this symbiosis, a nodular diagnosis was performed in field-sites chosen with farmers of the mediterranean basin, with common bean as a model grain-legume and major source of plant proteins for world human nutrition. With this methodology a large partial and temporal variation in legume nodulation was found in cereal-cropping and horticultural systems. In various reference areas of bean production, the nodular diagnosis confirmed that soil low P availability is a major limiting factor of the rhizobial symbiosis. The relation with legume improvement was further addressed by participatory assessment of bean recombinant inbred lines contrasting for their efficiency in use of phosphorus for symbiotic nitrogen fixation. Also in sites where low nodulation was presumably due to deficiency of native specific rhizobia, the inoculation with an efficient rhizobia increased nodulation and stabilized yield above 2 t ha^{-1} under irrigation. In some sites, within organic farming systems in particular, soil P bioavailability was found to be the highest in the rhizosphere of the most efficient symbioses. The later may be due to various mechanisms that are investigated in hydroaeroponics and rhizotrons, with emphasis on phosphatases, including phytase recently found in nodules. It is concluded that by increasing the phosphorus use efficiency for symbiotic nitrogen fixation, a virtuous cycle of fertility is activated within legume rhizosphere, which can contribute to the sustainability of agricultures through the use of appropriate legumes and cultural systems.

This work was supported by European Union projects Grain legume (CT-2004-506223) and Aquarhiz (CT-2004-509115)

SESSION 3

PROTEOMIC ANALYSIS OF SEED STORAGE PROTEIN DEFICIENCY IN COMMON BEAN

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[2] Department of Bioscience & Biotechnology, School of Life Sciences, Sun Yat-sen University, Guangzhou, China

Protein composition in mature seed was characterized by quantitative proteomics in two lines of common bean differing in seed storage protein composition. The SARC1 line contains the insecticidal protein arcelin derived from a wild accession (G12882). The SMARC1N-PN1 line lacks phaseolin, phytohemagglutinin and arcelin. The two lines share a similar level (ca. 85%) of Sanilac parental genetic background. The lack of major seed storage proteins in SMARC1N-PN1 is associated with a significant increase in sulphur amino acid content, particularly cysteine (1.7-fold; nmol per mg seed weight) and to a lesser extent methionine (1.1-fold), mostly at the expense of the non-protein sulphur amino acid, *S*-methyl-cysteine. The two lines share similar protein content, indicating a compensation for the absence of phaseolin, phytohemagglutinin and arcelin by otherwise minor proteins.

Protein composition was analyzed by two complementary quantitative proteomic approaches. Total protein extracts were submitted to SDS-PAGE, bands were excised, submitted to trypsin digestion and analyzed by liquid chromatography and tandem mass spectrometry. Quantification was performed by spectral counting, based on the total number of peptides assigned to a protein identified in the sample. Proteomic analysis was also performed after two-dimensional gel electrophoresis and quantification of protein spots by image analysis. The results of proteomic analyses were validated by a combination of Western blotting experiments and selective extractions. Selected major protein spots induced in the mutant line were analyzed independently by de novo sequencing of peptides and search for homologous proteins. Major changes in the accumulation of seed storage proteins were characterized, in particular compensation by residual lectins. Several proteins contributing to increased sulphur amino acid content in the mutant line were identified. Some of the differentially regulated proteins identified are involved in the metabolism of sugars and polysaccharides, and in protein synthesis, destination and storage. The results of these studies will be discussed.

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PHENYLPROPANOID PATHWAY GENE EXPRESSION PATTERNS ASSOCIATED WITH NON-DARKENING IN CRANBERRY BEANS (*PHASEOLUS VULGARIS*)

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Many of the coloured bean classes, including cranberry beans, darken after harvest, during storage. Because consumers associate darkened beans with poor quality they are priced lower in domestic and international markets (Singh et al, 2008). The current study was initiated to identify germplasm that is slow to darken and to identify the patterns of phenylpropanoid pathway gene activity that lead to the slow/non-darkening phenotype. A pale cranberry-like bean (Wit-rood boontje) was identified that darkened significantly less than conventional cranberry beans. From crosses made between the cranberry varieties Etna or Capri and Wit-rood F_1 and F_2 seeds with red stripes on a white background were selected and plants established from them were selfed. A screen of the resulting F_3 seeds identified several non-darkening cranberry-like lines as well as lines that darkened. Significantly lower levels of proanthocyanidins were measured in Wit-rood and nondarkening F_3 seed obtained from Etna x Wit-rood or Hooter x Wit-rood crosses than in Etna, Hooter or darkening F_3 seeds. In addition, the activities of F3H, DFR, LAR, ANR and VT, genes leading to the synthesis and accumulation of proanthocyanidins, were lower in the nondarkening seeds compared to those that darkened. The results suggest that the nondarkening trait in Wit-rood and its nondarkening progeny is related to reduced proanthocyanidin synthesis and indicate that the nondarkening trait can be incorporated into cranberry bean varieties.

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ARE *Lotus* SPECIES GOOD MODELS FOR STUDYING IRON ACCUMULATION IN COMMON BEANS?

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[2] iThemba LABS, Somerset West, South Africa
[3] Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Cracow, Poland.
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[5] Dept. of Agricultural Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark

Iron is an important micronutrient for all living organisms. Although iron is abundant in the Earth's crust, iron deficiency is a common problem throughout the food chain. Legume seeds have relatively high but also highly variable iron content. Little is known about the mechanisms controlling seed iron loading and only a few genes involved in seed iron metabolism have been described. Understanding the mechanisms that control seed iron loading and iron bioavailability can assist in breeding legumes with more bioavailable iron.

The aim of our study is to find *Lotus* genes responsible for the regulation of seed iron loading and plant iron metabolism, and use this knowledge to find the corresponding genes in Phaseolus species. For our studies we use the model legume *L. japonicus* for which large numbers of EST and genomic sequences are available, and the closely related *Lotus filicaulis* whose seed iron content differs from *L. japonicus*. Furthermore, we have recombinant inbred lines (RILs) from a cross between *L. japonicus* and *L. filicaulis* with good marker coverage.

In our study we have characterized mature seeds of *L. japonicus* and *L. filicaulis.* We have studied the distribution of iron in untreated mature seeds by simultaneously using Micro-PIXE and proton backscattering. In addition, chemical staining of iron in mature and germinated seeds was performed, and this material was studied by light microscopy before and after tissue fixation and sectioning.

Our studies show that the iron distribution in *L. filicaulis* seeds is similar to that in common beans, while the seeds of *L. japonicus* show a different pattern of iron accumulation. RILs from a cross between these two species are being studied in order to find genes that are important for seed iron distribution.

Furthermore, we are characterizing these two *Lotus* species in order to elucidate the physiological mechanisms behind the differential accumulation of iron in the aerial parts of the plants. We are studying the plants' ability to reduce the pH of the growth media, the iron reducing activity of their roots, and the effects of different concentrations of iron in the growth media on the plants' iron concentration.

Our studies indicate that *Lotus* plants are good model organisms for studying seed iron loading in legumes.

The work was supported by HarvestPlus, 2033 K Street, NW, Washington DC 20006-1002, USA and The Ministry of Science, Technology and Innovation, Denmark and the Research Foundation of the University of Aarhus, Denmark.

LOW PHYTATE BEANS IN THE FIELD: FIRST RESULTS

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Phytic acid, tannins and lectins, are considered the major antinutritional compounds of legume seeds, especially of bean seeds, which are largely consumed in developing countries. The reduction of their amount, could lead to an improvement of seed nutritional characteristics like increased mineral cation and phosphorus availability, and protein digestibility in the intestine of humans and monogastric animals. The CRA-Research Institutes of Montanaso Lombardo and Battipaglia, the CNR-IBBA of Milan and the University of Pavia (Dept. of Genet. and Microb.), carried out research programs focused on the development of innovative bean genetic materials characterized by the absence and/or reduced presence of such antinutritional compounds in the seeds.

First researches led to the development of lectin-free coloured- and white-seeded (this last correlated with low level of tannins) bean lines (Campion et al. 2008). Later on, the study on the reduction of phytic acid in bean seeds led to the development of a low phytic acid (*lpa*) mutant (*lpa*-280-10), which was obtained from an EMS mutagenised F_3 population of lectin-free seeds (Campion et al. 2009). All the most important features displayed by the seeds of this bean mutant were found to be highly positive: 90% reduction of phytic acid, seven times more of free or easily extractable (HCl 0.03 N) iron, 10 to 16 times more of free phosphorus, a very high germination capacity obtained using varying ageing or stress conditions and, finally, a very good field performance as judged by the data obtained in 2008 for the two most important agronomic parameters, seedling emergence (92% vs 89, 76 and 70% of controls) and dry seed yield (4.39 t ha^{-1} vs 5.28, 4.35 and 3.84 t ha^{-1} of controls). Considering that all the lpa mutants obtained so far in the other species (soybean, barley, rice, maize, wheat) through mutagenesis, present very serious physiological defects (low seed germination capacity, very low seedling emergence, stunted plant growth and very low seed yield), the optimal agronomic behaviour exhibited by our bean mutant was rather surprising. The confirmation of this apparent absence of physiological defects, was checked later in five (first cycle) cross-progenies (one from a lectin-free borlotto type bean and four from the lectin-free small white seeded line 586/8) made to introgress the *lpa* trait into different lectin-free bean genetic backgrounds.

Material and methods: a field trial was carried out in two field locations, one at Battipaglia (Salerno – south Italy), and the other at Montanaso Lombardo (Lodi – north Italy). Two hundred and forty seeds were sown according to a randomised complete block design with four replications. The CIAT lines BAT881 and A55, the lectin-free lines 938 and 586/8, the commercial cvs Sorano (Olter srl, Asti, Italia), Teggia, Etna and Dragone (Asgrow – Seminis Division – Vegetable Seeds Italia Srl), and the mutant line *lpa*-289-10 were used as the controls. In each experimental plot (3.00 x 0.60 m), formed by two rows, 60 seeds were sown (30+30). For each plot, the following parameters were examined: number and percent of emerged seedlings surveyed 26 days after sowing, dry seed yield (~13% water content) expressed as tha^{-1}, plant growth-period duration (no. of days from sowing date to harvest – surveyed only at Montanaso L.), average seed weight determined on a pool of 500 seeds. After skewness and kurtosis tests made to check the normality condition of data distribution, data were submitted to ANOVA analysis followed by "Duncan's Multiple Range Test" in order to rank yield means and compare their difference values at significance levels for $P \leq 0.05$ and $P \leq 0.01$.

Results: *lpa* progenies exhibited a very good seedling emergence (statistically equals or even better than the controls) and an acceptable seed yield that was depending on the location (in any case comparable to that of most controls). Also the average weight of the seeds produced by the *lpa* progeny deriving from the borlotto type parent showed a relevant gain in comparison to the mutant line *lpa*-280-10.

Research partially supported by Ministry of Agricultural Alimentary and Forest Politics with funds released by C.I.P.E. (Resolution 17/2003).

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POSTERS

PROTEOMIC CHARACTERIZATION OF ARCELIN AND PHYTOHEMAGGLUTININ SEED PROTEINS FROM *Phaseolus vulgaris*

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Arcelin and phytohemagglutinin are lectins seed storage proteins present in some wild bean accessions (*Phaseolus vulgaris*). They are implicated in the resistance phenotype of these wild beans towards the attack of coleopteran from the bruchidae family (*Zabrotes subfasciatus, Acanthocelides obtectus* and *Callosobruchus maculatus*).

A cost effective strategy is required in order to provide efficient and sustainable control of bean bruchids. A combination of genetic resistance, biological and cultural control methods can be effective. In efforts to develop bruchid resistant beans, researchers identified and introgressed different genetic resistance factors from wild relatives into common bean. Several authors describe the presence these proteins from the lectins family called insecticidal proteins (Phytohemagglutinins, PHA and Arcelin, Arc), appears to be the major resistance factors to control pest attack during storage on many varieties of *P. vulgaris*.

As a part of a national project for bean quality improvement in Venezuela, the present work show the identification by proteomic tools of Arcelin and phytohemagglutinin from commercial varieties of *P. vulgaris*, in order to complete the global analysis of these insecticidal proteins in seed varieties from Venezuela.

The seed material used in this work was (*Phaseolus vulgaris*) of the.The proteins were partially purified from mature seeds of kidney bean from Banco de germoplasma of INIA-CENIAP (Maracay-Venezuela) and characterized using proteomics tools as bidimentional gels and mass spectrometry. Total extraction of soluble proteins from bean endosperms of *P. vulgaris* was performed at pH 9. Bradford method was used for protein determination. Protein patterns from SDS-PAGE at 12% were stained with Coomassie brilliant blue, bidimensionals patterns on linear pH range from 3-10 followed by SDS-PAGE at 12% were developed by silver staining. The proteins were analyzed by MALDI-OTOF mass spectrometry.

The comparative analysis of the partially purified fractions among *P. vulgaris* proteomes revealed significant differences. The use of bidimentional gel exposed the specific insecticidal proteins for the MALDI-OTOF identification. These results appear to be a simplified procedure to initially establish functional correlations between these groups of proteins in these seed materials from Venezuela.

FIRST APPROXIMATION TO THE STUDY OF THE PROTEOME OF MESOAMERICAN AND ANDEAN BEAN GENOTYPES

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Proteomics has made it possible to identify a broad spectrum of proteins in different species. It is expected to revolutionize plant research, because it offers researchers new opportunities to increase the knowledge about both plant biology and crops improvement. This is unique technique not only for its ability to simultaneously separate thousand of proteins but also for detecting post-and co-translational modifications, which cannot be predicted from genome sequences. *Phaseolus vulgaris* has been suggested to be a model species to be investigated due to its selfish and synteny with respect to other legume species (Gepts et al. 2005). This situation points at bean as a good model to be analysed by proteomic methodology. This is the reason why we are initiating an approach to bean proteomics by two-dimensional electrophoresis (2-DE) with immobilized pH gradiens (IPGs). One of the critical problems that researchers have to take into account, when working in proteomics, is the low amount of proteins and the presence of some compound that can be interacting with proteomic protocols (Rose et al. 2004). In order to overcome these problems and go into the knowledge of bean extractome, it is necessary to establish which protocols of extraction are rendering the best results. With this purpose, leaves and seeds from two varieties representatives of both originary pools, Mesoamerican and Andean, are been analysed. Three different protocols of extraction are being used in both tissues and samples: TCA-acetone, Phenol and a commercial extraction kit (Clean-up Kit). The results show that there are appropriate protocols for each tissue of *Phaseolus vulgaris*.

Research was supported by the projects AGL2008-02091/AGR from the Spanish Government and INCITE07PXI403088ES form the Xunta de Galicia.

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DEVELOPMENT, TRANSFERABILITY, CHARACTERIZATION AND MAPPING OF SSR MARKERS ON THE COMMON BEAN REFERENCE POPULATION BAT93 X JALOEEP558

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As result of genome and cDNA sequencing projects of common bean, a vast amount of sequence information is becoming publicly available to be exploited for gene discovery and marker development. Simple sequence repeat (SSR) markers are among the most useful because of their great variability, abundance, and ease of analysis. Based on in silico analysis of 9,583 non-redundant expressed sequence tags (ESTs), 4,764 SSRs were found and 377 primer pairs were designed. A total of 315 SSRs (80%) were successfully amplified and 76 of them (24%) showed polymorphism between the genitors Bat93 (B) and Jalo EEP558 (J). The polymorphic EST-SSRs were mapped in the previously generated B x J linkage map using 75 F8 segregant individuals. One-hundred and eighty SSRs were located on this linkage map in addition to 123 previously mapped SSRs, forming 13 linkage groups (LGs) and spanning 1,154.5 cM, with an average marker interval of 6,6 cM. The EST-SSR markers were distributed over all the LGs. This SSR-based map will be useful as a reference map in common bean, facilitating the use of molecular markers in the breeding program. The transferability of SSR markers was examined among some Legume species. One hundred sixty seven SSR markers (102 genomic and 65 EST-SSRs) were used and the 82% were transferable for, at least, one of the species tested. Higher transferability index were achieved for species that belongs to *Phaseolus* genus (64%), followed by *Vigna* (26%), *Glycine* (29%), *Medicago* (10%), *Dipterix* (6%) and *Arachis* (2%). These transferable markers can now be exploited for further genetic and breeding studies in these species. The PIC was determined for a set of 167 SSR loci using 16 *P. vulgaris* accessions representing six commercial types of grains, and 72% of loci were polymorphic. The average PIC values ranged from 0.53 for genomic SSRs to 0.47 for EST-SSRs, with a mean number of alleles of 4 and 3, respectively. In the present study, a set of EST-SSR markers tested showed satisfactory levels of transferability, presented high levels of genetic content, comparable to genomic SSRs, and were integrated on a common bean linkage map, enabling their use in a broad range of applications regarding to genetic analysis of common beans.

Financial support: Prodetab, CNPq

INTERACTION OF WATER STRESS AND NODULATION IN BEAN LANDRACES

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Common bean (*Phaseolus vulgaris* L.) is often cultivated in unfavorable conditions and with minimal inputs. It is estimate that 60% of the bean crop is cultivated under risk of intermittent or terminal drought. The effects of drought on common bean are dependent on the intensity, type and duration of the stress. Due to the symbiotic nature of the legume-bacteria interaction for nitrogen fixation, effectiveness of the symbiosis to resist water deficit in the result of the combined capacity of both the plant and the specific rhizobial strain to cope with such stress. The existence of genetic variation in N_2 fixation response to water deficit among legume cultivars opens a real possibility for enhancing N_2 fixation tolerance to water deficit (TWD) through selection and breeding. The colonizing rhizobial bacteria also show marked variations in TWD. The objective of this work was coupling a selection of bean landraces that can be useful to improve TWD with the most efficient rhizobial strains. Ten common bean landraces and breeding lines from the MBG-CSIC bean collection were chosen and been inoculated each with ten different strains of *Rhizobium sp.* before planting in pots. Pots were subjected to two water regimes: more 90% field capacity (FC) and 60% FC. Two plants were harvested from each pot at the flowering stage, between 46 and 59 days after sowing, depending upon growth rate under greenhouse condition. Nodules were counted, and shoot and root separated. All components were dried at 70 ºC for 48 h to determine their dry masses. The experimental design was a randomized complete block with ten cultivars, four strains (reference strain *R. tropici* CIAT899 and three local strains EG, APAFI and LTMF), two water regimes and two replications. In dry environment PMB-0244, PHA-0155 and PHA-0683 had the best results. Drought stress reduced biomass yield by a range between 2% and 64 %. PMB-0244 had the highest yield in drought conditions and the lowest percent reduction. The local strains had better performance in drought conditions than the reference strain. The LTMF strain showed, in general, a high biomass yield (BY) and high nodules mass. The APAFI strain was the most efficient strain in drought-stressed environment it had a little nodule mass and a high BY. And finally, the EG strain was the less efficient with little nodule mass and BY. The couples with best performance in drought-stressed environment were PHA-0155 with EG, PMB-0285 with APAFI and PHA-0483 with LTMF. These cultivars could be incorporated into programs of genetic improvement for resistance to water deficit. These programs have to include a selection of strains, compatible with these cultivars, as a crucial strategy to improve the efficiency of rhizobia-bean symbiosis in drought-stressed environment.

This research was supported by the projects PGIDIT06RAG40301PR and INCITE 07PXI403088ES from the Galician Government.

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Phaseolus vulgaris ROOT RESPONSES TO *Rhizobium tropici* CIAT899 NOD FACTORS PRODUCED UNDER ABIOTIC STRESS

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Grain legumes are able to establish symbiotic relationship with a group of soil microorganisms collectively called rhizobia. The rhizobia-legume symbiotic interaction is a complex process that requires a sequence of highly regulated events, involving the coordinated expression of both plant and bacterial genes. Nod Factors are produced and secreted by rhizobia in response to plant exudates. Nod factor perception by the plant induces several morphological and physiological changes in the roots which are essential for successful nodulation. *Phaseolus vulgaris* is considered a poor N_2 fixing grain legume, is a promiscuous host nodulated by at least six species of rhizobia. One of these species is *Rhizobium tropici* that is highly stress resistant. It grows under acid conditions, in large amounts of salt, and at high osmotic pressure. An earlier study reported a substantial effect of acid stress on qualitative and quantitative production of Nod factors (Morón *et al*. 2005). The aim of the present work was to investigate the effect of high salt (NaCl) concentrations on Nod factor production and biological activity of these Nod factors on *Phaseolus vulgaris* root. For this purpose HPLC and mass spectrometry analyses were carried out. *Phaseolus vulgaris* does not grow with more than 25 mM NaCl. Therefore the biological activity of the Nod factors produced by *R. tropici* CIAT899 under high salt was tested without salt, which was already removed in the purification procedure of the Nod factors. To investigate the biological activity of the under stress produced Nod factors, we tested the 45% and 60% acetonitrile fractions yielded by SPE (solid-phase extraction) of the crude extracts of the Nod factors produced under neutral, acid stress (pH 4.5) or salt stress (300 mM NaCl) conditions. They were similar in biological activity, inducing root nodule-like structures (pseudonodules) analogous to those observed on the *Rhizobium*-inoculated plants (positive control). The 20% acetonitrile SPE fractions, which after HPLC separation and Mass Spectrometric analysis did not appear to contain Nod factors, were not active.

This work has been supported by grant AGL2006-13758-C05-AGR

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SENSORY EVALUATION OF NEAR ISOGENIC LINES DERIVED FROM THE FABADA MARKET CLASS A25 LINE

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The sensory value of dry beans (*Phaseolus vulgaris* L.) is one of the traits that most influence consumer's choices. It is often argued that the process of genetic improvement might reduce the sensory value of dry beans through negative genetic correlations among traits or through the remnants of the donor genome that are interspersed with the recipient genome through backcrossing. However, there are no experimental data to document these theoretical changes.

Since 1990, breeding programs have been underway in Asturias to improve the fabada commercial type (very large (100g/100 seeds) white oblong seeds) by incorporating genes to improve resistance to anthracnose (genes *Co-2* and *Co-9*), BCMV (gene *I*), BCMNV (gene *bc-3*), and to modify the architecture of the plant (gene *fin*). Near isogenic lines (NILs) obtained from the A25 line, derived from individual selection in the fabada-type commercial variety Andecha, are currently available. Lines A2806 (*Co-2*+*I*+*bc-3*) and A2418 (*I*+*bc-3*) were obtained by backcrossing with A25 as the recurrent parent. The characteristic indeterminate growth pattern of the fabada type was modified by the cross V203 x A25 to obtain the determinate-growth Xana line. Later, genes that brought resistance to anthracnose were incorporated into Xana by backcrossing, to obtain the line X1319 that contains the gene Co-9 and the line X1358 that contains the gene *Co-2*.

To identify possible differences in the sensory quality of these NILs, line A25 and the lines derived from it A2806, A2418, Xana, X1319, and X1358 were cultivated in two locations (Argüelles and Villaviciosa, Asturias, northern Spain). The sensory characteristics of the seeds harvested were evaluated by a trained panel. Significant differences between the two locations were detected for '*roughness of the seed coat surface'*, '*seed coat perception*', and '*mealiness'*. On the other hand, no differences were found in the trait '*flavor'*. Only the trait '*mealiness'* had a significant *location x variety* interaction.

Xana and its derivative NILs were different from A25 and its derivative NILs in the trait "*roughness of the seed coat*". No differences in the trait '*seed coat perception'* were observed between Xana and A25; however, the seed coats of lines X1319 and X1358 derived from Xana were more perceptible than the rest. Xana was less mealy than the other lines, all of which fell into a single group of significance. Finally, lines X1319, A2806, and A2418 had a less intense flavor than A25.

The results indicate that there are sensory differences in the NILs derived from the breeding program, although these differences do not necessarily result in worse quality. The changes identified may be due to remnants from the donors, recombination, epitasis or pleiotropy.

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IRON, ZINC, AND FERRITIN ACCUMULATION IN COMMON BEANS

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Iron and zinc malnutrition are major threats to human health and development around the world. The World Health Organization states that over two billion people are affected by iron deficiency. In particular children and pregnant women in developing countries are affected by iron deficiency. A common nutritional base for poor populations is a staple such as maize, wheat, rice, potatoes, cassava, or beans, but many of these have low iron and zinc content as well as potent inhibitors of iron uptake. Nutritional supplements are often unavailable to such populations due to lacking infrastructure, education, and funding. A sustainable alternative to nutritional supplements is to cultivate plants that have a higher content and bioavailability of nutritionally important elements in their edible parts. HarvestPlus is a challenge program whose aim is to develop and distribute new cultivars of staple crops with increased nutritional value.

In spite of the discovery of several genes involved in the regulation of iron and zinc metabolism in plants, the key regulators for seed iron accumulation have not yet been described. Little is known about the molecules that are responsible for chelating iron in mature seeds, but the ferritin protein was suggested to be the major iron storing protein in legumes [1].

Both iron and zinc localization, as well as speciation, can have an impact on their nutritional availability. We will present detailed information about iron, zinc, and ferritin distribution in common beans. We used micro-PIXE (Particle Induced X-ray Emission) and proton backscattering analysis to localize and quantify zinc and iron in mature bean seeds. In addition the iron distribution in different *P. vulgaris* genotypes was studied using Perl's Prussian blue staining. We show that the distribution of iron is dependant on the genotype.

Using immunolocalization, we visualized the localization of ferritin in mature common bean seeds.

This knowledge can contribute to the discovery of factors that affect the bioavailability of micronutrients and can contribute to breeding common beans with increased nutritional value.

The work was supported by HarvestPlus, 2033 K Street, NW, Washington DC 20006-1002, USA and The Ministry of Science, Technology and Innovation, Denmark and the Research Fundation of the University of Aarhus, Denmark.

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DIFFERENCES IN THE PHENOLIC COMPOSITION OF SOME *Phaseolus* VARIETIES

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Beans (*Phaseolus*) are a source of dietary protein in general, and particularly for vegetarian population. They contains several phenolic compounds, representing an important group of bioactive compounds in legumes [1], which exhibit strong antimutagenic, antioxidant and antigenotoxic activities [2, 3].

In this work the phenolic composition of five different beans varieties were studied; two colored, one pink-mottled cream and two whites. Phenolic compounds were extracted by maceration in methanol-HCl/water. The obtained macerated were purified and then ,analysed by HPLC-PAD-MS, agree with the method of Dueñas [4].

The analyzed beans contain hydroxybenzoic acids, gallic, protocatechuic and p-hydroxybenzoic and hydroxycinnamic compounds, *p*-coumaric and ferulic acids, and derivatives of them. Also flavonols, and flavanones have been found.

In the pink mottled cream and colored beans have been identified, in addition, flavanols (monomers and dimers) and a high number of quercetin and kaempferol derivatives, dihydroflavonols and flavanones (pinocembrin, eriodictyol and naringenin derivatives). Also anthocyan glucosides and derivatives (mainly cyanidin and pelargonidin derivatives) were identified. These flavonoid compounds are abundant in the colored samples.

In the white beans the most abundant compounds are hydroxycinnamics (mainly ferulic acid derivatives) in higher concentrations than the rest of phenolic compounds

The differences observed in the phenolic composition of beans it is important because, the beneficial health activity attributed to these compounds depends on the chemical structure of said antioxidant; non-flavonoid compounds, hydroxybenzoics and hydroxycinnamics, have less antioxidant activity than flavonoids [5].

The colored beans have larger amounts of flavonoid compounds, especially of flavanols (proanthocyanidins) and anthocyans, which could contribute to a higher antioxidant activity than the white beans.

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GENETIC DIVERSITY AND TECHNOLOGICAL TRAITS OF A COMMON BEAN LANDRACE FROM SICILY (ITALY): FAGIOLO A BADDA

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Historical investigations provided evidence of the long-lasting tradition of common bean (*Phaseolus vulgaris* L.) cultivation in the Italian peninsula as well as in its major islands. This implies a long story of adaptation of this crop to Italian agro-environments. The main result of this process is constituted by the selection of a myriad of local common bean populations. Nowadays, only a part of the original autochthonous germplasm is still cultivated in small areas mainly located in marginal environments. In the last decades several Italian common bean landraces have been investigated to promote their safeguard, valorisation and conservation. Recent collecting missions carried out in the Sicily have evidenced that this region still conserves in its marginal areas a lot of common bean landraces.

The present study was undertaken to characterise the common bean landrace traditionally cultivated at Polizzi Generosa (PA, Sicily) named Fagiolo a Badda. This name is usually used by local farmers to indicate a group of local populations all characterised by bicolour seeds that can be divided in two sub-groups Badda nero (from violet/cream to black/cream seed), Badda bianco (orange/cream seed). Nine accessions, five for Badda nero and four for Badda bianco were used in this study. The genetic diversity within and among the accessions was evaluated by using molecular markers of SSR type (microsatellites) following the methods described in Lioi *et al.* (2005). The dendrogram based on Nei's genetic distance provided the following indications: the Badda bianco bean accessions were clustered together demonstrating high genetic similarity. Conversely, the Badda nero bean appears to be constituted by two well-distinguished sub-groups.

In order to estimate the technological performances of these accessions the following parameters were investigated: cooking time, coat percentage, hydration and swelling indices according to the methods described in Piergiovanni *et al*. (2000). Relevant differences among the accessions belonging to the same sub-group were recorded for the hydration index and cooking time. By combining molecular and technological data obtained in this study, we found that an appreciable level of genetic diversity exists within as well as among Badda accessions managed by Sicilian farmers.

Research supported by Ministry of Agriculture Food and forestry policies with funds released by C.I.P.E (Resolution 17/2003).

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PODS MORPHOLOGICAL EVALUATION OF BULGARIAN SNAP BEAN RILS REGARDING MECHANICAL HARVEST AND PRODUCT QUALITY

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Garden bean (*Phaseolus vulgaris* L.) is widespread in almost every country and it is one of the traditional Bulgarian crops. Mechanical harvest of pod production is enabled only on varieties with determinant growth habit and simultaneous pods development. Furthermore, “French-type” varieties with green, cylindrical, long, strait and slender pods are preferable for canning and freezing. Uniformity of maturation is a variety specific trail, and production of one -time mechanical harvest represents total of pods with different maturation level regarding their organoleptic characteristics and market quality.

Therefore, the purpose of this work was complex study of pods morphology of garden bean RILs, and also to identify those ones which suits specifically for both uses: (i) one-time mechanical harvest and (ii) processing.

Twenty garden bean RILs with oval pods and determinate growth habit and one standard-variety from the collection of “Maritsa” Vegetable Crops Research Institute in Plovdiv were chosen for the experiment. The following parameters were evaluated for each of them: pod length (PL, cm), pod width (PW, mm), index of pod straightness (IPS, %), index of oval- shaped pods (IOP, %). Classification of the RILs was made, using criterion proposed by Silva and Antunes (2003). Mean pod samples were made be method of Manuelyan, 1966.

Two classes for pod length were determined: ten RILs with intermediate PL (10 to 11.55 cm) were longer than standard variety; and ten RILs were with short pods (<10 cm).

Three classes for pods width were determined: four RILs with very slender pods (<7.84 mm), fourteen RILs and standard- variety with intermediate pods (7.84 to 10.42 mm), and two RILs with very wide pods (<10.42 mm).

Indexes of pod straightness and oval- shaped pod define the superior quality of the canned product. The highest are indexes, the straighter and cylindrical the pods are. Depending of the IPS studied RILs were classified as follows: ten of them and standard-variety were very straight (IPS>0.9%), and next ten were straight with IPS from 0.85 to 0.9%. Depending of the IOP studied RILs were classified as follows: eight of them and standard-variety were with cylindrical pods (IOP>0.9%), and twelve RILs were with oval pods with IOP from 0.7 to 0.9%.

The mean values of the morphological trails were determined and lines with higher values were selected. Research results showed that 86% of the evaluated RILs are suitable for making a first class of chopped pods or hole-piece cans. Such characteristics as erect plant type, uniformity of maturation, and desirable first pod insertion, associated to intermediate to long-sized, cylindrical, strait and slender pods without grain development, are well suited to mechanical harvest and to achieve good product quality with high commercial value.

This document was created with the financial support of Human Resources Development Operational Programme, co-financed by the European Union through European Social Fund. Document's content is at the sole responsibility of Maritsa Vegetable Crops Research Institute and it could not be considered as an official statement of European Union and Contracting Authority under any circumstances.

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CARBOHYDRATES AND INORGANIC ION CHANGES DURING SALT STRESS EXPOSURE OF *Phaseolus* SPECIES: INVOLVEMENT ON GROWTH, WATER RELATIONS AND OSMOTIC ADJUSTMENT

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Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semiarid region of the world, reducing the value and productivity of the affected lands. Soil infertility is often due to the presence of large quantities of salt and the introduction of plants capable of surviving under these conditions is worth interesting. Currently, there are no economically viable technological means to facilitate crop production under salt stress conditions. However, development of crop plants tolerant to salinity stress is considered a promising approach, which may help satisfy growing food demands of the developing and under-developed countries. Development of crop plants with stress tolerance, however, requires, among others, knowledge of the physiological mechanisms and genetic controls of the contributing traits at different plant development stages. We evaluated the contribution of soluble carbohydrate content (total hexoses and sucrose) and inorganic ion accumulation in the osmotic adjustment of four *Phaseolus* species under increasing salinity. The experimental system was characterized through the analyses of several parameters known to be strongly affected by salinity, such as relative growth rate (RGR, g g^{-1} day), unit leaf rate (ULR, g m^{-2} per day), leaf area ratio (LAR, m^2 g^{-1}), specific leaf area (SLA, m^2 g^{-1}), leaf weight ratio (LWR, g leaf g^{-1}), gas exchange, water relations, and rate of ion uptake. These variables were calculated for the period between 10 and 20 days after planting. Salinity significantly reduced RGR, ULR, LAR, and SLA but did not affect LWR. In salt-tolerant species, ULR, but not LAR, was significantly correlated with RGR, indicating that ULR was an important factor underlying the salinity-induced differences in RGR among species. In salt-sensitive species, high salinity reduced SLA, and consequently LAR. The rate of CO_2 assimilation decreased gradually with salinity, showing significant reductions only at the highest salt level (90 mM NaCl). The reduction of CO_2 assimilation rate was attributable to reduced stomatal conductance and increased leaf Cl^- content. Leaf water and osmotic potentials declined significantly as stress conditions intensified. However, positive turgor was maintained or enhanced throughout the entire growth period. Salinity caused a progressive increase in hexose in leaves and roots of *Phaseolus* species, mainly in the salt-tolerant. Hexose accumulation moderately contributed to osmotic adjustment and, thus maintaining turgor pressure at 60 mM NaCl. In contrast to soluble carbohydrates, total inorganic ions formed the largest component contributing to osmotic adjustment in stressed *Phaseolus* species under salt level. Salinity had a significant effect on tissue concentration and uptake of Na^+, K^+ and Cl^-. Thus, in addition to the toxic effects of high concentrations of Na^+ and Cl^- in plant tissue, salinity induced changes in mineral nutrient uptake likely contributed to the reduction of plant growth. Our results suggest that the salt tolerance in wild *P. vulgaris* and cultivated *P. acutifolius* was able to maintain a more adequate osmotic pool in the leaves and roots under salt stress than the salt-sensitive species.

RESEARCH CONCERNING THE PHENOTYPIC VARIABILITY OF SOME GARDEN BEAN HYBRIDS (*Phaseolus vulgaris L.*)

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The paper presents the studies of F1 hybrids resulting from diallel crosses and the parental forms according to Griffins model. According to this method, we selected parental forms with different origins: 4 certified varieties (Carson, Jutta, Inka, and Lingua di Fuoco) and 3 local populations from Brăila county (Tichileşti, Vădeni and Movila Miresii).

It were obtained following hybrid combinations: H1 - Inka ♀ x Carson ♂; H2 - Inka ♀ x Jutta ♂; H3 - Jutta ♀ x Carson ♂; H4 - Lingua di Fuoco ♀ x Carson ♂; H5 - Lingua di Fuoco ♀ x Jutta ♂; H6 - Vadeni ♀ x Lingua di Fuoco♂; H7 - Inka ♀ x Vădeni♂; H8 - Lingua di Fuoco ♀ x Movila Miresii♂; H9 - Tichileşti ♀ x Carson ♂; H10 - Movila Miresii ♀ x Carson ♂. The study of F1 and F2 generations was carried out on the field at Vădeni, from Braila county, in 2007 and 2008 years, where is a calcaric aluviosol type of soil. We studied the productive indices of the average number of pod per plant, average length of pods, the average number of beans in the pod, and in terms of production quality was analyzed crude protein content of pods at technological maturity and of grain at physiological maturity, determinations made by Kjeldahl method.

The phenotypic variability of the main quantitative and other morphologic and physiologic proprieties were studied for each genotype.

The experiment was done using the method of randomized blocs with 3 repetitions. Each experimental lot had 25 plants. The results assessment was performed using the statistical row of observations method and the variance analysis.

The objectives envisaged for the purpose are:

- variability analysis of morpho -physiological characters of biological material from the original collection, evaluation and selection
- evaluation of genitors for controlled hybridization of varieties, hybrids and populations studied, so as to obtain hybrids with high genetically potential in terms of qualitative and quantitative production and for resistance to biotic stresses (diseases and pests) and a biotic (drought, soil fertility etc.).
- the general combination ability (GCC), a specific combination ability (CCS) and identify the degree of correlation with the morphological and physiological parameters for productivity.
- variability analysis of morphological and physiological characters of the hybrids obtained compared with the parental genotypes.

The greatest growth of heterosis parents to the media concerning the number of pod per plant, was obtained for 5 of hybrids in the F1 generation, while in the F2 generation had heterosis effect 7 of hybrids and are recommended improvers for their inclusion in the breeding of garden beans. In the South - East of Romania there is a great potential for growing french-beans, explaining the reason that during the collection of biological material originally found a large number of cultivation with a morpho-physiological variability pronounced.

MOST CO- LOCI OF COMMON BEAN COULD BE MADE UP OF CLUSTERS OF RACE-SPECIFIC ANTHRACNOSE RESISTANCE GENES

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Currently, thirteen anthracnose resistance genes, designated as *Co-* (*Co-1* to *Co-13*), have been described in common bean. Some of these genes have been located in the integrated linkage map, either by direct mapping or indirectly using molecular markers: *Co-1* on linkage group B1, *Co-2* on B11, *Co-3* and *Co-9*, demonstrated to be allelic (*Co-3/Co-9*), located on B4, *Co-4* on B8, *Co-5* on B7, *Co-6* on B7, and *Co-10* on B4. Recently, an anthracnose resistance gene provisionally designed as *Co-u* has been mapped on B2.

Broad genetic variability for *C. lindemuthianum* has been described worldwide, with more than 100 different races of the pathogen identified. Most of the *Co-* genes were described as single genes conferring dominant resistance (except *co-8*) to several anthracnose races. However, in agreement with the cluster organization of families of resistance gene analogue sequences (RGAs) and/or resistance gene candidates (RGCs), mapping close to some of these genes, genetic analyses of joint segregations for resistance to different anthracnose races demonstrated that some of the *Co-* genes are organized as clusters of individual genes conferring race-specific resistance.

In this work we analyze the segregation for resistance to several races of anthracnose in the RILs proceeding from the cross between the breed line Xana and the anthracnose differential cultivar Cornell 49242, and in a population of F3 families obtained from the cross between the anthracnose differential cultivars Kaboon and Michelite. Molecular marker analyses were carried out in these populations in order to map and characterize the anthracnose resistance genes or gene clusters present in Xana, Cornell 49242 and Kaboon.

The results indicate that:

1 Two loci conferring resistance to anthracnose, located in linkage group B4, corresponding to *Co-3*/*Co-9* and *Co-10*, respectively, have been found in Xana. Both loci are formed by clusters of different race-specific resistance genes: *Co-3*/*Co-9* includes at least four different resistance genes against races, 9, 65, 73, and 1545, respectively, and *Co-10* includes at least four different resistance genes against races, 3, 7, 19, and 449, respectively.

2 One locus conferring resistance to anthracnose, located in linkage group B11 and corresponding to *Co-2*, has been found in Cornell 49242. This locus is made up by a cluster of at least 9 different resistance genes conferring specific resistance to races 3, 7, 6, 19, 38, 39, 65, 357 and 449, respectively.

3 Two loci conferring resistance to anthracnose, located in linkage groups B1 (*Co-1*) and B4 (*Co-3/Co-9*), respectively, have been found in Kaboon. In this differential cultivar, locus *Co-1* confers resistance to race 81, and locus *Co-3*/*Co-9* is a cluster including at least three different genes conferring specific resistance to races 3, 7 and 19, respectively.

The results obtained add further evidence to previous reports supporting the possibility for most Co- loci to be composed of clusters of different race-specific anthracnose resistance genes.

CHARACTERIZATION OF RESISTANCE LOCI TO THE ANTHRACNOSE PATHOGEN (*Colletotrichum lindemuthianum L.*) IN ANDEAN AND MESOAMERICAN BEANS

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Common bean (*Phaseolus vulgaris* L.) is considered one of the world's most important legumes, and anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Msgn.) Bri. & Cav., is a major disease of the crop, causing losses of up to 100% (Schwartz, 1991; Beebe and Pastor-Corrales, 1991). Co-evolution is known to have occurred between fungal pathotypes and the common bean's two gene pools (Balardin and Kelly 1998; Melotto et al., 2000). Congruent with this co-evolution, the best sources of resistance for breeding programs are often found in complementary gene pools. To assess the potential impact of both pathogens on the snap and dry bean Spanish production, 47 snap bean cultivars and 137 breeding lines obtained from a total of 23 dry bean cultivars were inoculated with Andean races 17, 73, 448 and 1545 and Mesoamerican races 7, 23, 39, 55 and 102 of anthracnose. To further evaluate the effectiveness of the markers in selecting for resistance to be used in plant-breeding programs, it was compared the markers and observed phenotypes for bean cultivars inoculated with isolate of anthracnose. This report extends our knowledge of the virulence of pathotypes of *C. lindemuthianum* to Andean and Mesoamerican genetic material in Spain. In general, the Andean and Mesoamerican pathotypes infect Andean and Middle American germplasm, respectively. The independent evaluation of races 7, 23 and 55 showed a relatively high level of susceptibility in germplasm for both gene pools. Race 73 presented a high percentage of susceptibility in Andean accessions compared to the Mesoamerican accessions, while race 1545 had a pathogenity similar in both gene pools. The reactions of the genotypes to the nine pathotypes of *C. lindemuthianum* tested provide valuable information for breeders and farmers.

Research was supported by AGL2008-02091/AGR and INCITE07PXI403088ES projects from the Spanish Government and Xunta de Galicia, respectively. M. De La Fuente and A.M. González acknowledge fellowships from the Xunta de Galicia that allowed them to carry out this study. Special thanks to J.D. Kelly for anthracnose inocula.

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