

The PROVESOS Research Group: Sustainable Plant Production

Activities in 2016



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The Group of Applied Research on Sustainable Plant Production (PROVESOS, by its initials in Spanish) maintains its official recognition by the Government of Aragon (Spain) since December 31, 2015. Currently renewed until 2016 since the moment of its creation in 2002.

The Group is composed of researchers and technical staff (see members) belonging to the Units of Horticulture and Plant Protection from the Food and Agriculture Research and Technology Center of Aragon (CITA), the Dept. of Agricultural Sciences and Natural Environment of the University of Zaragoza (Polytechnic School of Huesca), the Center of Plant Health and Certification (CSCV) and the Plant Technology Unit (UTV) of the Department of Rural Development and Sustainability (formerly Agriculture and Livestock) of the Government of Aragon. Most of the researchers are integrated into the newly created Food and Agriculture Institute of Aragon (IA2) (CITA + University of Zaragoza). The Group also has a technical-scientific collaboration within the business sector of fertilizers and plant protection products (Dr. **Begoña Martín-López** from Adama Agriculture S.A.).

During the past two years the Group has experienced an important process of renewal for the retirement of most of its senior members and the incorporation of new researchers and technicians. Thus, in November, 2014, **Sonsoles Fernández-Cavada** (agronomist, CSCV) is retired, and two students for training finished the contracts with the CITA: **Pablo Monge-Blasco** (Grad. in Biological Sciences) and **Ana Isabel Marí-León** (agronomist). Fortunately the latter rejoined the activity of the Group in May 2015 as technical staff.



On the other hand, the researchers Dr. **Aurora Díaz-Bermúdez** (biochemist) (CITA), **Santiago Fuertes-Lázaro** (agronomist, CSCV) and **Antonio Carranza- Pérez** (Agric. Eng., UTV) joined the Group, as well as Dr. **Ana M. Sánchez-Gómez** and Dr. **Vicente González-García**, starting its activity in the Units of Horticulture and Plant Protection, respectively in 2015. Likewise, Dr. **Carlos Zaragoza-Larios**, former Co-ordinator of PROVESOS, has been appointed to CITA as Associate *ad honorem* Doctor and re-incorporated to the Group.

Thus the PROVESOS Group is composed of specialists in various disciplines related to plant production and health (agronomy, genetics and plant breeding, biotechnology and molecular biology, entomology, bacteriology, virology and weed science), who work in scientific research centers (CITA and University) and dedicated to the management and technological support to the regional agricultural sector (CSCV and UTV), and provide the Group one of its main strength: its remarkable multidisciplinary character, ensuring adequate flow knowledge along the chain of research, development and transfer of results.



GENERAL OBJECTIVES OF PROVESOS RESEARCH ACTIVITY

The overall objective of the activity of PROVESOS Group is to contribute to the development of plant production methods that make it compatible economic viability by reducing its environmental impact, focusing on aspects such as characterization, conservation and appropriate use of plant genetic resources, and optimization of plant protection techniques based on knowledge of the dynamics of agricultural ecosystems, and biological and integrated control of harmful organisms, including pests, diseases and weed flora. The activities of this research group generally are structured in the following lines of research:

A. Characterization, conservation and enhancement of plant genetic resources

- A.1. Conservation, characterization and management of germplasm of horticultural species.

- A.2. Characterization, regeneration and upgrading of traditional vegetable varieties and improved varieties grown mainly in Aragon.



- A.3. Analysis of biodiversity, identification of genes of interest related to quality, efficiency in resource utilization and resistance to pests and diseases, and its use in breeding programs aimed at obtaining new varieties of horticultural interest.

B. Effective and sustainable strategies in plant protection

- B.1 Design fast and reliable methods of diagnosis on emerging and re-emerging plant diseases.



- B.2. Identification, taxonomy, evaluation and characterization of pests, diseases and weeds currently threaten productivity and quality of economically important crops in Aragon, mainly cereals, alfalfa, olive and fruit orchards.
- B.3. Study the life cycle of pests, their natural enemies, pathogens and weed flora, and analysis of ecological and epidemiological factors (biotic and abiotic) that determine their distribution and proliferation.
- B.4. Design new strategies for biological and integrated pest, disease and weed control, their integration in the work of crop management, and evaluation of their impact on the agricultural ecosystem (weed flora, wild insect fauna and birdlife).

As part of these lines of research, the focused research activities carried out by the PROVESOS Group during the past year are detailed, and subsequently the indicators of the Group's activity relative to their participation in research projects, scientific information generated, agreements with other entities, and related dissemination and training activities are briefly described.



DESCRIPTION OF THE RESEARCH ACTIVITY OF PROVESOS

RESEARCH AND SPECIFIC ACTIONS DEVELOPED

A. Characterization, conservation and enhancement of plant genetic resources

A.1. Characterization, conservation, and management of germplasm of horticultural species

Some indicative figures of multiplication activities, characterization and management of plant genetic resources carried out during 2015 in the Zaragoza Horticultural Species Germplasm Bank (BGHZ) as part of its ongoing activity are listed here:

- Conservation activities Plant Genetic Resources BGHZ for long-term maintenance.
- Updating information National Inventory (IN) and BGHZ database.
- Sending duplicates to the base collection Plant Genetic Resources Centre of INIA(CRF, Madrid).
- Feasibility study of preserved samples. Germination tests of 124 entries belonging to 9 species (between 24 and 28 years of conservation).
- Successful regeneration of 177 entries (139 in the CITA, 10 in the EPSH and 18 Rijk Zwaan seed company with which a cooperation agreement was established). These inputs have become part of the active collection of BGHZ, being available to users, and a sub-sample is sent to the base of CRF collection. Furthermore, primary characterization data and photographs on most of these entries have been obtained, which provide added value to the collection and ease to use. They have also been taxonomically classified those inputs from which the species was unknown.
- 177 regenerated samples include more than 25 vegetable species (onion, leek, chard, borage, cabbage, cucumber, pumpkin, zucchini, carrot, lettuce, bean, pea, field pea, radish, tomato, eggplant, spinach, pepper, watermelon and melon) and, to a lesser extent, wild species (*Taraxacum officinale*, *Silene vulgaris*, etc.).

- Inclusion of 81 new entries to the active BGHZ collection. These samples have been collected and re-generated by the Plant Genetic Resources Centre of INIA (CRF, Madrid).
- Attention to the seed requests to BGHZ by interested users (researchers, breeders, farmers, etc.) during the period 2014-2015 (until June), there have been a total of 71 shipments, in which 895 samples have been sent. The species include: tomato (sent 108 entries), onion (82), squash (77) and peppers (64). All of these requests have been addressed through the signing of a material transfer agreement.
- Maintenance and improvement of the web application with information about the BGHZ, with option seeds request on line: <http://sites.cita-aragon.es/BGHZ/introduccion/>



A.2. Characterization, regeneration and upgrading of traditional vegetable varieties and improved varieties grown in Aragon (Spain).

The following activities are mainly carried out under transfer activities (agreements, contracts and demonstration projects), which have focused on the following species and varieties:

- **Tomato ‘Rosa de Barbastro’**. In order to evaluate the performance of the selections they have been made corresponding demonstration trials during the 2015 campaign. The results confirm the good performance of the two selected lines and have initiated contacts with the industry to transfer the seed obtained. The characterization of tomato ‘Rosa de Barbastro’ plant material has allowed the registration as a conservation variety at the National and Community catalog, being the final registration date on 02/19/2015.
- **Onion ‘Fuentes de Ebro’**. Demonstration trials have been conducted with farmers to study the behavior of the selected line onion ‘Fuentes de Ebro’, evaluating productive and quality aspects, including hot taste. Considering the results obtained in the next campaign aims to cultivate plots selected large-scale farmers seed.

- **Bean ‘Caparrona de Monzón’.** Tests were carried out in the field, in the experimental plot of CITA and Monzón, which have allowed the characterization of plant material of this variety. The obtained data have helped to define the bean ‘Caparrona’ by morphological and phenological parameters, and agronomic interest; and obtain quality seed in sufficient quantity to enable the sector to address its production and marketing and preserve seed in the Germplasm Bank to ensure their long-term maintenance.



• **Onion cultivation techniques.** It is readying a new growing technique of the onion ‘Fuentes de Ebro’, in collaboration with a company of the sector.

- **Local varieties.** It is collaborating with the project "Sobrarbe undertakes" developed by the Sobrarbe region (Huesca, Spain), through technical advice and supply of plant material from local vegetable varieties for the establishment of pilot farms.
- **Carrots.** It is developing the phenotypic characterization of local cultivars of carrot, from the BGHZ, which differ in color (orange, white, yellow, purple).



• **Lettuce.** In addition, we are working in the preparation and edition of the project entitled "Biofortification of lettuce (*Lactuca sativa* L.) using local varieties and wild relatives as a source of variability and classic, genomic and biotechnological tools," which has been presented in the Program RTA 2015 of Spanish INIA and, in relation with the above line, plans to address the

phenotypic characterization of germplasm of lettuce: commercial varieties, traditional varieties and wild relatives, with the following specific actions:

- Quantification of vitamin C content in local varieties of lettuce with potential commercial interest.
- Quantification of anthocyanins content in commercial varieties and traditional varieties of lettuce with potential commercial interest, as well as wild forms of the same gender related.
- Quantification of anthocyanins in local varieties and wild accessions under water stress conditions.



A.3. Analysis of biodiversity, identification of genes of interest and genetic improvement of horticultural species

During 2015 specific actions have been carried out within the framework of the research indicated:

- Molecular study of the mechanisms involved in resistance to *Fusarium oxysporum* race 1.2 f. sp. *melonis* in **melon**: work has continued by optimization for detection by RT-PCR of this fungus that causes Fusarium wilt in this crop and plant death in infected melon plants.
- Search for sources of resistance to Fusarium wilt in watermelon: it has begun development of a protocol for *Fusarium oxysporum* artificial inoculation f. sp. *niveum* watermelon.
- Application of analytical methodologies that allow precise phenotyping, associated with the presence of nutraceutical compounds in the ***Capsicum* and *Allium*** species: analytical method HPLC-MS / MS has been applied for detecting capsiate, nutraceutical compounds only present in the fruits of genus *Capsicum* in different cultivars. This methodology is very useful to identify these compounds and observe the variability of study materials.

- Study of genes or gene sequences involved in the biosynthesis of capsinoids: it has begun to study the allelic variability of ALMPs, one of the enzymes involved in the capsiate biosynthesis pathway. This study is allowing the identification of the alleles of cultivars and species PamT until now studied and assess its relationship with the synthesis of these compounds.
- Development and application of molecular markers in breeding of horticultural crops: we apply molecular markers being implemented in **onion** developed for the study of male sterility in populations of this crop.
- In phenotypic characterization local **carrot** (*Daucus carota* L.) cultivars, from the BGHZ (Germplasm Bank), it has started studying metabolites with strong antioxidant activity, such as carotene and anthocyanins in different accessions of carrot comprising both commercial varieties ('Orange' and 'Purple'), as local varieties (with a variation in color ranging from white to purple, through yellow and orange). It is expected to carry out the genotyping of these accessions by using molecular markers, as well as analysis of the expression of genes involved in the synthesis of metabolites of interest, both in different tissues, as in samples from field trials with different location (environmental impact study).
- Within a national research project which we are working in collaboration with the group of Genomics in Plant Breeding (Dr. A.J. Monforte) of the Institute of Molecular and Cellular Biology of Plants (IBMCP), validation field tests of introgression lines (ILs) of **melon** has been conducted. To do this, we have evaluated the fruit from approximately 200 plants belonging to 13 different families, along with the recurrent parent ('Piel de Sapo') for traits of interest in breeding, such as shape, weight, content sugar, etc.



Regarding the Biofortification **lettuce** project, presented at the INIA-RTA2015 national program, it is predicted to be addressed in relation to this research:

- The identification of genes responsible for the increased anthocyanin production in lettuce:
- Identification of genes involved in anthocyanin biosynthesis pathway that are differentially expressed in several accessions and in different organs of the same

plant.

- Identification of genes involved in anthocyanin biosynthesis pathway that are differentially expressed in control conditions and under water stress.
- Search polymorphisms in genes with differential expression and its regulatory regions that explain the anthocyanin enhanced content.
- Introduction of alleles responsible for a high content of anthocyanins in landraces rich in C vitamin by cisgenesis.

B) Effective and sustainable strategies in plant protection

B.1. Design and optimization methods of diagnosis diseases fast and reliable

- Development of a new protocol for the detection of *Xanthomonas arboricola* pv. *pruni* by magnetic immunocapture and real-time PCR.
- Performing an international ring-test for the evaluation of two protocols *Xanthomonas arboricola* pv *pruni* detection recently developed (real-time PCR and LAMP)
- Development of real-time PCR technic for detection of *Xylella fastidiosa* bacterium quarantine (quarantine pathogen in the EU) recently detected in Europe and serious threat to many crops of economic interest in Aragon.
- Development of a protocol for the detection of *Lonsdalea quercina* subsp. *populi* (subsp. nov.) in poplar.
- It has continued the work aimed to obtain a diagnostic method using nested RT-PCR of rhabdovirus causing excrescences in alfalfa, which also cause reduced plant growth and reduced crop persistence syndrome.

B.2. Identification, taxonomy, evaluation and characterization of pests, diseases and weeds

Entomology

- Study of potential pests with high population densities (Orthoptera, Acridoidea)
- Identification and evaluation of insect pests and beneficial insects (parasitoids and predators) more important in the olive groves of Aragon

- It has begun a collaboration to contribute to the study of taxonomy of insect pests of the order Coleoptera. For it has signed a collaboration agreement with the University of Agriculture in Shenyang (China) that will allow the predoctoral student Mr. Chuan Bu Gao, the Insect Taxonomy Laboratory of Plant Protection College of this university, to make a two year stage starting in 2015 in the Entomology Laboratory of the CITA to complete his doctoral thesis under the co-direction of Dr. M. M. Coca. This agreement may lead in the future to further exchanges of scientific personnel between the two institutions.

Bacteriology

- Studies of the biology and epidemiology of the quarantine bacterium *Xanthomonas arboricola* pv. *pruni* in *Prunus* spp., and monitoring of the phytosanitary status of *Prunus* species in the Aula Dei Campus.
- Assessment of losses due to *X. arboricola* pv. *pruni* in commercial plantations of almond in Aragon.
- Whole genome sequencing of a pathogenic strain of *X. arboricola* isolated pattern plum *Prunus mahaleb* in Aragon.
- Studies on the varietal plum and almond sensitivity to *X. arboricola* pv. *pruni* in commercial plots in Aragon.
- Possible first identification in Spain of the bacteria *Londsdalea quercina* subsp. *populi* (subsp. nov.) and *Brenneria populi* (sp. nov.), causal agents of cankers in poplar. Surveys have been done to study the incidence of these diseases in Aragon.
- Monitoring and analysis of the incidence and epidemiology of alfalfa mosaic (AMV) and viruses that cause alfalfa excrescence virus in the Ebro valley.
- Identification in samples of alfalfa in the province of Zaragoza of a virus causing excrescences belonging to the genus Capulavirus, proposed new genus within the family *Geminiviridae* virus in samples of alfalfa. This new genre could include the first virus of this family transmissible by aphids.
- Characterization of isolates of rhabdovirus causing excrescences in alfalfa by obtaining partial sequences of L gene viral replicase.



Weed research

- Monitoring and control of patches of the invasive weed the yellow nightshade *Solanum elaeagnifolium*, located on roadsides and fallow of various areas in Zaragoza, Caspe and Quinto de Ebro (Aragon, Spain) since 2005.
- Evaluation of the problems caused by the presence of teosinte (*Zea mays*) in maize in Aragon. Teosinte, the wild maize, is a new weed that affects the cornfields of the Ebro valley. Although the official Plant Protection Center (CSCV) have been aware of its existence at the end of the summer of 2014, it is known that the first infestations appeared about already in 2009. In 2015 we have conducted surveys on the affected areas and having briefings in different parts of Aragon in coordination with the Plant Protection Service of the neighbour Catalonia to spread awareness of this weed and prevent new infestations. There has been 41 fields were surveyed and subsequent samples and data processing on the possible presence of teosinte into maize fields in Aragon. In the municipalities of Candanos, Peñalba and Torralba de Aragón (Huesca) and Bujaraloz, La Almolda and Ejea de los Caballeros (Zaragoza) from September 2014 to January 2015.
- They have held regular meetings of technicians from CSCV and researchers at the CITA with the Spanish Plant Variety Office (OEVV) of the Ministry of Agriculture and Environment (MAGRAMA, the National Association of Plant Breeders (ANOVE), the Agricultural and Agri-food Cooperatives, the Agri-food Regional Offices of Aragon, the Associations of Integrated Treatment in Agriculture (ATRIAS) and the Agricultural Professional Associations to analyze the problem of teosinte in Aragon from September 2014 to November 2015.
- Attention has been paid to questions on suspected weeds to be resistant to herbicides. Tests for performing dose-response curves with different herbicides to confirm resistance and testing and chemical control alternatives have been made. The CSCV have had working meetings with various entities concerning the problems of teosinte:
 - With technicians ATRIAS extensive cultivation of Aragon: “Emergence of a new weed in the crop”. Almudevar (Huesca) September 11, 2014
 - Plant Protection Network in Aragón: “The problem of teosinte into maize plots cultivated in Aragon”. CSCV, Zaragoza, September 15, 2014.
 - With the Servei de Sanitat Vegetal (Plant Health Service) from the

Department of Agriculture, Livestock, Fisheries and Food of Catalonia.
“Problematic of teosinte in Aragon and Catalonia”. Fraga (Huesca). September
16, 2014.



B.3. Study of biology and ecology of pests, their natural enemies, pathogens and weed flora, and the factors that determine their distribution and proliferation.

Pests

Study the biological cycle of orthoptera as potential pests, the olive borer *Hylesinus toranio* and their parasitoids

Estimation of environmental factors to explain the spatial variation and behavior of the plague of Mediterranean lobster (*Dosciostaurus maroccanus*) (Orthoptera, Acrididae) and other potential pests ortópteros.

Identification of environmental factors inductors of variability in the phenotypic expression, cline variation in phenotypic traits of the *Chorthippus biguttulus* group (Orthoptera, Acrididae).

Study the biological cycle of *Hypera postica* (Coleoptera: Curculionidae) and *Colaspidema atrum* (Coleoptera: Chrysomelidae) on commercial plots under different management practices.

Diseases

Assessment of the survivability and seed transmission of *X. arboricola* pv. *pruni* in almond.

Study of the genetic diversity of Spanish strains of *X. arboricola* pv. *pruni*.

Studies aggressiveness of a collection of *X. arboricola* pv. *Pruni* Spanish strains.

Preparation and characterization of plant pathogenic bacteria isolated from fruit and vegetable from diverse backgrounds for use in genetic diversity studies, epidemiology and control. Maintenance and expansion of a reservoir collection and Spanish strains in the Collection Française de Bactéries Phytopathogènes (CFBP).

Estimating of the non-persistent transmission capacity of various strains of alfalfa mosaic virus (AMV) by aphids and evaluating possible mechanical transmission capacity by cutting.

Analysis of genetic diversity of isolates AMV transmitted by seed and virus isolates obtained over the cropping period of alfalfa (four years) in fields of Aragon.

Maintenance and conservation of rhabdovirus isolates causing excrescences in alfalfa by graft transmission and persistent vectorial transmission.

We have worked in the preparation and drafting of the project entitled "Virus Diseases of Alfalfa: 1) effects of integrated epidemiological parameters and control the evolution

of diversity mosaic virus (AMV), 2) etiology and characterization of viruses that cause enations "presented at the national program INIA RTA 2015. In connection with this research, the project will address the comparative study of transmission associated with AMV factors and their relationship to the evolution of genetic diversity in alfalfa fields under two systems of pest control: conventional and integrated management.

Weeds

Study of the biology of teosinte (*Zea mays*) under the conditions of Aragon. Research has focused on:

- a) The tests to determine the maximum depth of seed emergence.
 - b) The analysis of the viability of the seeds in the medium term, burying at different depths and determining their viability every 6 months
 - c) To know the maturity dates and prolificacy of teosinte plants in the field.
- d) Study the possible effect of other crops on the emergence of teosinte in the greenhouse and field.

B.4. Design strategies biological and integrated pest, disease and weed flora control and evaluation of their environmental impact

Pests

- Study sex pheromones of aggregation and oviposition of Mediterranean locust (*D. maroccanus*). Estimation of pheromonal activity in the field for the locust integrated control.
- Effect of cutting alfalfa winter break in the density of population dynamics of eggs and larvae *H. postica*. Impact on production, quality and persistence of the crop.
- Effect of cut alfalfa in winter latency in the incidence of parasitoid larvae of *H. postica*.
Incidence of parasitoids in eggs of *H. postica*.
- Effect of the presence of alfalfa uncut strips in the population dynamics and the level of damage caused by *Colaspidema atrum* in alfalfa.

Diseases

- In the last year we had a field experience with products that can reduce the incidence of bacterial fire blight (*Erwinia amylovora*): biological products, mainly resistance inductors and nutritional. The results have not yet been analyzed.

Weeds

- Tests have continued with biodegradable materials in pepper mulching for weed control (INIA project). Trials have been initiated with biodegradable materials developed by the company Nurel (Zaragoza) for mulching in horticultural crops and a commercial paper for mulching from Stora Enso Company (Finland) has been tested on a particular farm.
- Trial of methods for teosinte control in fallow (tillage, herbicides) (INIA emerging project) and effect of rotations (ANOVE agreement).
- Implementation of the resolutions of the General Director of Food and Agribusiness Development (Agriculture Department) for precautionary control measures for the eradication of the teosinte established on certain fields of Aragon. January to October 2015.
- Conducting field surveys (863 field plots and 7,080 ha) to verify compliance with mandatory measures for the control of teosinte and for monitoring the evolution of the infestation and identification of possible new outbreaks. May to November 2015.
- Assessment of a weed control test in the line of culture by the erosion caused by the impact of air powered pellets from agricultural waste.



• Identification and integrated management of barnyard grass (*Echinochloa* spp.) and sprangletop (*Leptochloa* spp.) infesting rice, prevention of resistance and cultural management methods. We have continued prospecting rice plots in monoculture and in rotation with other crops, as fescue and winter cereals, that in previous campaigns were sown with rice to evaluate the influence of rotation in the presence of *Echinochloa*. We have started collecting seeds of barnyardgrass possibly resistant to herbicides (INIA project approved in 2015).

- Study of the resistance of species of the genus *Conyza* spp. to the herbicide glyphosate in Aragon populations (Peñaflor (Z), Fraga (Hu) and Maella (Te)).
- Study of the *Rapistrum rugosum* resistance in winter cereal to the sulfonylurea herbicides group.
- Impact of *H. postica* and *C. atrum* control strategies in the composition and abundance of weeds in alfalfa.



Photo: PROVESOS members in 2010