

Instituto Juan March de Estudios e Investigaciones

11

CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Lecture Course on Conservation and Use of Genetic Resources

Organized by

N. Jouve and M. Pérez de la Vega

R. P. Adams

R. W. Allard

T. Benítez

J. I. Cubero

J. T. Esquinas-Alcázar

G. Fedak

B. V. Ford-Lloyd

C. Gómez-Campo

V. H. Heywood

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P R O G R A M M E
CONSERVATION AND USE OF GENETIC RESOURCES

Monday, February 22nd

General Addressing and Introduction. N. Jouve.

- M. Pérez de la Vega - Techniques for Genetic Diversity Evaluation.
- F. Orozco - Domesticated Animals: Problematic in Conserving Breeds.
- T. Benítez - Evaluation and use of Microbial Resources.

General Discussion.

- J.T. Esquinas-Alcazar - Institutional Aspects: the FAO Global System for the Conservation and Utilization of Plant Genetic Resources and the Convention on Biological Diversity.
- T. Hodgkin - Using Gene Banks Better: Structured Sampling and Core Collections.
- V.H. Heywood - Conservation of Germplasm of Wild Species in Europe.

General Discussion.

Tuesday, February 23rd

- D. Zohary - Wild Genetic Resources of Cultivated Plants in the Mediterranean Basin and the Possible Strategies for Their *in Situ* Conservation.
- J.I. Cubero - Germplasm Collections. What We Learned and what We Can Learn from Them.

Poster Session 1.

- G. Fedak - Transfer of Alien Genetic Variation in Plant Breeding.

General Discussion.

Visit to the Germplasm Bank of Cruciferae, E.T.S.I.A, Madrid.

- C. Gómez-Campo - Plant Conservation: the Case of Wild Species.

Wednesday, February 24th

- R.P. Adams - DNA Conservation and the DNA Bank-Network.
- B.V. Ford-Lloyd - *In Vitro* Conservation of Genetic Resources.
- Poster Session 2.
- L. Navarro - Conservation of Genetic Resources of Woody Species with Recalcitrant Seeds: Problems and Perspectives.
- General Discussion.
- R.W. Allard - Methods of Identifying Useful Germplasm.
- C.O. Qualset - Plant Genetic Resources Evaluation and Gene Transfer.
- J.W. Snape - Chromosome Engineering and Gene Transfer from Wild to Cultivated Cereals.
- General Discussion and Concluding Remarks.

INTRODUCTION

N. Jouve

Nicolás Jouve.
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The Course deals with the Conservation and Use of Genetic Resources. As we move into the twenty-first century it is very important for Geneticists and breeders all over the world to assess carefully what has already been achieved, what is the current status of our genetic resources, and to suggest what the future is likely to hold. Besides affecting many people, genetic resources conservation has many aspects that, ranging from basic and applied research to political and institutional decisions, can influence legal questions, commercial interests, etc. The course will cover all of this in a condensed form, but, it will center its attention on the characterization and use of genetic resources.

During the last years, some scientific circles have spread the dream that major benefits from living beings will soon be forthcoming from the use of genetic engineering and biotechnology. Molecular Biology has arrived on the center stage of scientific and public-policy debates. The new techniques in cell and tissue culture, DNA manipulation and transfer, are all being touted as the scientific answer for increasing disease and pest resistance, tolerance to chemicals, good quality or other performances, and consequently increasing the yield potential of food crops and domestic animals.

Breakthroughs in molecular biology and biotechnology are occurring with increasing speed. Meanwhile, the main future options are being foreclosed by the erosion of one of the mankind's important legacies, the genetic diversity of living organisms: particularly microbes, domestic animals and crop plants and their wild relatives. Moreover, it is necessary to direct our attention to Genetic Resources and balance the tendency towards a narrowing genetic base of our crops, which has intensified in recent years because of the widespread use of standard lines, or by the extensive use of selected varieties that have been spread successfully over the world.

Our objective is to review methodologies and the status of the exploration, collecting, characterization and evaluation, conservation and use of the Genetic Resources represented by crop plants and their wild relatives, domesticated animals and microbes. Genetic Resources represent the raw material with which applied geneticists and breeders work. The bottom line is germplasm conservation now in preparation for future applications that benefit humanity.

Particular attention will be given to plant genetic resources, because cultivated plants and their related wild and weed species have received much more attention from geneticists and breeders than animals and microbes.

The Program of the Course has been ordered grouping into five Sessions related topics. These range from the analysis of the variability in each kind of organism to the particular application of genetic resources in breeding.

The first Session analyzes variability and tries to answer the following questions: how much variability is there in a species or population?; what kinds and amount of variability should be preserved?; how much of the variability is really relevant?; how can we

preserve genetic diversity in Animals (in live animals, in embryos, by cryopreservation, in DNA)?; how can we evaluate the genetic diversity in natural populations of microorganisms?. These questions are explored in the lectures on: 'Techniques for Genetic Diversity Evaluation', 'Domesticated Animals: problems or problematic in conserving breeds', and 'Evaluation and use of microbial resources', given by Dr Pérez de la Vega, Dr Fernando Orozco, and Dra Tahía Benítez, respectively.

The lectures of the second session review international actions and institutional cooperation for the conservation of genetic resources. Some of the questions treated are the following: how must be organized the germplasm collections in appropriate agro-ecological conditions?; what is the present on intergovernmental discussions on the availability of germplasm collections?; how can we best explore and collect genetic resources?: how can we select a representative and useful genetic spectrum of diversity for our 'core collections' from large existing collections?; how can we manage large seed collections, species with recalcitrant seeds, or in vitro tissue samples?; what are the actions towards an EC genetic Resource Programme?. Dr José Esquinas reviews the aspects of International Cooperation in his lecture 'Institutional Aspects: The FAO World System and the Convention on Biodiversity'. The organization of a plan to co-ordinate conservation in wild species in Europe is treated by Dr Vernon Heywood, in his lecture 'The European Programme on Wild Species Relatives to Cultivated Plants'. Finally, the principles guiding the modern collections of crop germplasm are discussed in the lecture 'Using genebanks better: structured sampling and core collections', which is presented by Dr T. Hodgkin.

The task of preserving genetic resources is one that concerns the whole of mankind, and it is not restricted to just those species that we presently exploit. Something which can be readily appreciated with regards to wild species that are most effectively preserved in their natural state. This task and the strategies to make the germplasm collections widely known, adequately preserved and effectively used could be summarized in the following questions: how can we best preserve the sources of variability?; how can we make the existing variability available for future breeding programs?; how can we manage in situ conservation?; what did we learn and what can we learn from germplasm collections?. The strategies for in situ and ex situ conservation, the gene bank conditions needed to conserve the germplasm collections, and the importance of wild species in broadening the genetic base of crops, is the focus of the third Session. This includes the lectures 'Wild genetic resources of cultivated plants in the Mediterranean Basin and the possible strategies for their in situ conservation', 'Germplasm collections. What we learned and what will learn from them', and 'Transfer of alien Genetic Variation in Plant Breeding', which are given by Drs Daniel Zohary, Jose Ignacio Cubero and George Fedak, respectively.

The course also includes a visit to the Cruciferae Germplasm Bank, at the Agricultural School of the Madrid Polytechnical University. There, Dr Cesar Gómez Campos will give a lecture on the methods of long-term seed conservation used in the unique and valuable Cruciferae collection that he founded, with the help of the Juan March Foundation.

The fourth session is devoted to other alternatives for long-term conservation of biological resources. We must remember that Genetic Resources are not exclusively linked to conventional plant or animal breeding research. They are also of general importance

for future needs concerning the use of genetic engineering and molecular methods. Up to now, we have only used vertical gene pools in our breeding programs. The recent advances in Molecular Biology open new perspectives in which DNA can be transferred between distantly related species, even between different genera. New methods yet available and used to conserve genes or germplasm material are DNA conservation, in vitro cell-culture, cryopreservation and embryo manipulation. All these alternatives are treated in the fourth session, which also deals the following questions: How can we structure and manage a DNA Bank-Network in specific programs for horizontal gene transfer?; is in vitro preservation an alternative to field genebanks?; what techniques exist for in vitro germplasm storage?; how can we preserve woody species with recalcitrant seeds?;. In his lecture on 'DNA conservation and the DNA-Bank network', Dr Robert Adams outlines recent advances in DNA technologies and the establishment of Nodes in the DNA Bank net. Recent advances in in vitro conservation including slow growth storage and cryopreservation are explained by Dr Brian Ford-Lloyd and Dr Luis Navarro, in the respective lectures 'In vitro conservation of genetic resources', and 'Conservation of Genetic Resources of woody species with recalcitrant seeds: problems and perspectives'.

In the final Session, is reviewed practical use of genetic resources. The following questions are discussed: what kind of genetic traits can be used to identify useful germplasm?; what is the most convenient framework for evaluation of Plant Genetic Resources and gene transfer?; how can we incorporate alien variation into cultivated species by chromosome engineering?. These topics are covered in the following lectures: 'Methods of identifying economically useful germplasm', 'Plant genetic resources evaluation and gene transfer', and 'Chromosome engineering and gene transfer from wild to cultivated cereals'; which are explained by Dr Robert W. Allard, Dr. Calvin Qualset, and Dr. John Snape, respectively.

Without doubt, studies on Genetic resources involve a broad range of research activities, They include population genetics, cytogenetics, pathology, ecology, numerical taxonomy, molecular biology, statistics, etc., All these specialties are usually present in the modern gene banks. The present course attempts to approach the methods for storing variation in living organisms. We cannot pretend that we presently have the technical answer to all the complex questions related with Genetic Resources. On the contrary, we can only hope to offer an introduction to the actions, which are being taken to make genetic resources available and safe. Secondly, we can show how at least some of the many existing problems can be solved.

The directors of this course Dr Marcelino Pérez de la Vega and Dr Nicolas Jouve, thank the Scientific Committee of the Centre for International Meetings on Biology of the Instituto Juan March de Estudios e Investigaciones, on the behalf of the Genetical Society of Spain, which proposed this Course, and particularly to Mr Andrés González, his Director for his energetic input in organizing the activities of this prestigious Centre. They also express gratitude to the select list of the lecture speakers who by accepting the invitation to participate in the course guaranteed its success, and given all the participants the opportunity to learn from them during the three days of the course.

MONDAY, FEBRUARY 22nd

Techniques for genetic diversity evaluation

M. Pérez de la Vega; Universidad de León

During this course, I am sure that among other questions a question will be arise repeatedly: What kind and amount of genetic variability should be preserved?. This question is intimately related to two other questions: How much variability is there in a species or population? and, how much of this variability is relevant?. This presentation is mainly focussed on the methods to evaluate genetic variability, and in particular on those biochemical or molecular methods to do so. Likewise, some evidence of the usefulness of the assessment of "molecular" variability will be given.

Genetic diversity within populations and within species determines the rates of adaptive evolution and the extent of response in traditional crop improvement. Natural and artificial selection choose among the variants that occur within populations, based on their adaptation to the immediate environment or their fitting to the breeder's interest. The goal for crop improvement is to agronomically fix useful genetic variants within cultivars by selective breeding. Therefore, breeders, conservationists and evolutionists are concerned with the extent and quality of genetic variability.

The traditional approach to characterization and evaluation of populations involves morphological and agronomic description. Considered as a whole, numerous morphological data are difficult to comprehend in terms of patterns of variation in populations. For this reason, numerical taxonomic techniques are needed to simplify and handle these complex patterns of variation. Traits of agronomic interest such as vigor, disease resistance and cold tolerance, and so on, are usually under high genotype-environment interactions. Morphological evaluation of population variability may be supplemented and generally surpassed by a more direct study of the genome by means of the analysis of biochemical markers. These markers, in particular isozymes, have been extremely useful in improving our knowledge of the genetic composition of populations and for determining the magnitude of various evolutionary forces involved in molding the genetic architecture of plant populations. In the future, DNA polymorphism studies will be a further and definitive step in this knowledge. Biochemical markers are less affected, if any, by environmental factors and numerous data can be handled and statistically analyzed in terms of patterns of genetic variation, at least those traits such as isozyme and DNA polymorphisms whose genetic control is easily understood.

Several kinds of biochemical markers have been used for the characterization of plant populations. These markers can be grouped in three classes:

- 1) A heterogeneous pool of biochemical compounds including phenolics, alkaloids, cyanogens and non-protein amino acids, that can be designated as low molecular weight markers.
- 2) Proteins, including both enzymes and storage proteins.

3) DNA markers, including fragment of variable length obtained by digestion with restriction enzymes (restriction fragment length polymorphism, RFLP), DNA polymorphisms shown by PCR, and base sequences.

In this presentation the techniques and the relevance of the first group of markers will only be briefly commented on. It will be devoted to describe and compare the usefulness and limitations of protein/isozyme electrophoretic techniques, still the most widely used technique in the estimation of genetic variability in plants species and populations, and the techniques to analyze DNA polymorphisms whose use is increasing exponentially. In particular DNA fingerprinting by random amplified polymorphic DNA (RAPDs) is being rapidly introduced into the method for genetic variability assessment.

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DOMESTICATED ANIMALS: PROBLEMATIC IN CONSERVING BREEDS

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In a general review some basic principles are presented, such as: The reasons for preservation of definite populations - especially breeds -, as well as the causes for the disappearance, or danger of loss, of many breeds of domestic animals. Some points concerning the concept of breed and of other sub-populations within the species, and the contrast of losing a breed compared with that of a species. Interest to preserve specific populations or genetic pools, its fundament. Difficulties to preserve animal compared with plant material. Economical and sociological problems involved in conservation. Differences between the problematic in developed or developing countries.

Technical and scientific aspects of the breeds conservation are specially treated, with the corresponding references for more ampliation if needed. Basically, it is being considered the problem of maintaining the genetic variability to prevent the deterioration of small populations and to preserve the pretended special characteristics of each breed. Population size, level of inbreeding and heterozygosity, loss of specific genes, etc., are some topics of main concern.

It follows a brief review of some ways to preserve genetic material other than by maintaining live animals: cryopreservation technologies for gametes, embryos, stem cells and segments of DNA; with reference to their intrinsic limitations.

It is included a review of the most important ways or systems to carry out the conservation of populations of live animals belonging to endangered breeds. Programmes supported by public or private founds, with their advantages and disadvantages or limitations in both cases, and information drawn from their results during the last years; the more positive approach being that with programmes run by both types of support, with diverse roles played by each side. Convenience of participation of the grower sector: livestock farmers or fancier breeders associations. Type of organizations in charge of conservation programmes, at regional, national and international level, and their convenient collaboration. Different programmes according to species, mainly in basis to their cost and management, or in basis to the animals included being "for accompaniment", "ornamental", "hobby", etc., or somehow productive.

Some examples of programmes now in action with proposals for new ones, plus comments on the actual situation of the problem, are finally reported.

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EVALUATION AND USE OF MICROBIAL RESOURCES

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The contribution of the microorganisms to the overall genetic pool is enormous; there are authors who assume that each species of arthropod or vascular plant supports at least one species of nematode, one of protozoon, one of bacterium and one of virus as parasites; it has also been told that the proportion of fungal species with regards to vascular ones is six to one; even that the consequence of ten-fold reduction in length implies that the number of species which fits in such range of size increases one hundred-fold.

The diversity observed in natural populations of microorganisms is considerably higher than that known in the usual laboratory cultures. Among bacteria, there are species able to fix N_2 or CO_2 , oxidise sulphides or methane, reduce CO_2 to methane, metabolise xenobiotic compounds or digest macromolecules with extracellular enzymes; other species are parasites of animals or plant pathogens or symbionts; some others are able to grow at very high salt concentrations, pHs lower than one or higher than twelve, temperatures higher than 110° or in the presence of ionising radiations of high intensity. The genetic diversity within the same species is extremely high in the streptomycetes, and reaches its maximal values in some groups of halobacteria where the probability of a cell to be genetically identical to its parent is of only 80%.

Similarly, yeasts can be isolated from soils from the Tropics to the Polar Regions, from salted or fresh waters, from organic debris, mainly vegetal detritus, from the surface of fruits or the cuticle of insects, from the skin of animals where they live as commensals or pathogens, etc. Yeasts can metabolise an enormous variety of substrates which includes xylose, cellobiose, lactose and other sugars, hydrocarbons and alcohols, xylan, pectic substrates and phenolic compounds. Yeast applications include their use as bakers' yeasts, single cell protein and food production, lipid or ethanol production, or the production of beer, wine, distillery, lactic compounds, glycerine, vitamins, amino acids, enzymes, polyhydric alcohols and carotenes, among many other compounds.

Since not all yeast strains are able to mate, the classification of yeasts into species has been carried out according to certain physiological features such as the yeast capability to ferment and/or assimilate different substrates. Recently, the interfertility found between strains which had previously been unable to conjugate, has allowed different species to be gathered and classified now as a unique species, as for example it happens with *Saccharomyces cerevisiae*. However, under an industrial point of view, each of the different species of *Saccharomyces* which are now classified as *Saccharomyces cerevisiae* has been associated with a specific fermentation

process so that, for practical reasons, the former classifications and names are still being employed.

Together with morphological and metabolic tests, techniques of molecular biology are recently used as a good complement to the classical techniques to classify yeasts. These techniques include electrophoresis of extracellular fractions, studies on the protein profiles, profiles of fatty acids of long chain, polymorphism of DNA sequences, chromosome electrophoresis and analysis of the restriction maps of mitochondrial DNA. These techniques allow to distinguish strains at inter- or intraspecific levels, and therefore to differentiate those strains of the same species which have been isolated from the same ecological environment. The knowledge about the genetic diversity within the same species allows us to guess how far a species threatened with extinction is or is not in the non-return way: when a species is about to be extinct, it has already lost most of its genetic diversity. On the other hand, this diversity has led in many cases to the formation of strains with improved features after constructing hybrids between non-isogenic parental strains.

The application of, first, techniques of molecular biology to establish phylogenetic relationships existing among yeasts; second, the exploitation of the intraspecific variability existing in natural population; and third, the construction of hybrids between non-isogenic strains have been successfully applied to wine yeasts isolated from different Spanish regions. With regards to the first case, phylogenetic relationships among wine yeasts isolated from regions as different as Rioja, La Mancha, Alicante, Jerez or Albariño have been established, or autoctonous yeasts from musts from Majorca, Canary Islands or Galicia have been characterised. In relation to the second point, the contribution of each of these yeasts to the flavour, organoleptic and analytical features of the different wines has also been established. Finally, with regards to the third point, the increase in ethanol tolerance with respect to their parents, of those hybrids formed between non-isogenic parental strains, isolated from different Spanish musts and already highly ethanol-tolerant has also been established. From these hybrids, there has been carried out a selection in continuous culture controlled by pH of those hybrids able to tolerate and produce the highest ethanol concentrations, in order to be used in ethanol production as an energy source from appropriate substrates as the carbon source.

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INSTITUTIONAL ASPECTS: THE FAO GLOBAL SYSTEM FOR THE
 CONSERVATION AND UTILIZATION OF PLANT GENETIC RESOURCES AND
 THE CONVENTION ON BIOLOGICAL DIVERSITY

Owners and users of germplasm are not necessarily the same. In addition, most of the existing genetic diversity is to be found in the tropical and sub-tropical areas where a large number of developing countries are located, while most modern technologies and economic capacity to utilize germplasm are to be found in the industrialized countries of the North. No country or region can be self-sufficient in its needs on genetic diversity; according to recent studies, the average crop genetic resources dependency (crop production relying on exotic germplasm) among regions of the world is more than 50 percent, and for some regions it may go up to 100 percent for the most important crops. Local information and traditional technologies developed by farmers and farmer communities for their specific varieties and species are also of utmost importance for a better understanding and wise utilization of this germplasm. This is, therefore, an area in which all countries are at the same time donors and recipients and where international cooperation is a vital imperative.

Furthermore, the collection of germplasm, as well as its rejuvenation, characterization and evaluation in appropriate agro-ecological conditions can only be done through international cooperation and adequate agreements among the countries involved. Exchanges of germplasm and related information and technology should ideally involve all countries of the world. Pioneers in promoting international technical cooperation in this area have been the Food and Agriculture Organization of the United Nations (FAO) since the fifties and the International Agricultural Research Centres (IARCs) of the Consultative Group (CG) since their establishment in the seventies. Numerous technical meetings and publications and an increasing number of projects and activities on plant genetic resources have been hosted, edited or financed all over the world through these organizations.

However, questions regarding international cooperation are not only technical or financial. The last few years have seen a growing acknowledgement of the greatly increased value of plant germplasm, due to the fact that rapid genetic erosion has shown that germplasm is not an unlimited or replenishable resource, and to new biotechnologies that have greatly expanded the frontiers of its utilization. This has already resulted in a number of formal or practical restrictions on the availability of germplasm. In addition, questions such as the safety of the material, the ownership of collections and the development of national laws restricting the exportation of certain species or protecting intellectual property rights for new varieties, became the subject of continuing debate. Since the relative value of plant genetic resources will continue to grow rapidly in the near future, it has become clear that plant germplasm needs to be protected for the use of future generations, and its availability for scientific purposes ensured through equitable international agreements and regulations that guarantee governmental commitments.

Intergovernmental discussions on these policy and legal matters, that started in FAO in 1979 have resulted in the negotiation and approval of (i) the 1983 FAO International Undertaking on Plant Genetic Resources with its complementary annexes (two annexes were approved in 1989 and a third one in 1991), which has led to the development of the FAO Global System for Conservation and Utilization of Plant Genetic Resources; and (ii) the 1992 UNEP-negotiated Convention on Biological Diversity, which also covers Plant Genetic Resources.

The evolution, context, coverage and perspectives of these agreements will be discussed during the lecture.

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Using gene banks better: structured sampling
and core collections

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National gene banks must not only cover all phases of germplasm activities (from collection to fostering the use of variation), they must also operate with limited resources. Core collections, which consist of a limited set of accessions derived from an existing collection, chosen to represent the genetic spectrum in that collection, can assist gene banks to carry out their tasks and provide users with the genetic diversity they need for research, plant breeding and other tasks. Core collections will usually contain about 10% of the collection, but may well be a larger fraction of small collections and provide the user with a set of genetically and ecologically distinct accessions.

The general scheme for setting up a core involves four operations. These are (1) data assembly, (2) grouping of like accessions, (3) selection of the entries for the core from each group, and (4) handling of the entries. Each step raises questions that require a specific answer in each project, but a general approach can be developed through the use of structured sampling procedures. In addition, decisions must be made about two further operations, namely the revising of the core and the collecting of new samples. Multivariate methods and sampling theory provided some principles and assistance in these decisions.

As well as providing a way of managing large seed collections, core collections can also assist gene banks handling clonal crops, or species with recalcitrant seeds. Field gene banks are expensive to run and vulnerable to loss. Options to lessen these problems include the storage of tissue samples *in vitro*, or, in some species such as sugarcane and tuber crops, the storage of seed. The core approach offers a way to choose accessions for growing in the field, for the development of new methods and for monitoring conserved samples.

Core collections have met with criticisms in four main areas: (1) that the rest of the collection is vulnerable to decay or disposal, (2) that the bias to representing diversity ignores usefulness, (3) that the system is too inflexible, and (4) that variation within accessions is ignored. Although these concerns often reflect misuse or misunderstanding of the core approach, they raise issues which need to be carefully considered.

The principles of stratified, representative sampling in the core concept also apply to the choice of populations for conservation *in situ*. By adopting sampling methods that ensure coverage of the genetic and ecogeographic ranges of a species, better use of limited resources will enable specific scientific goals (such as the discovery of new resistances) to be achieved. The same gene sampling theory indicates that such modest projects would capture the majority of target alleles.

Some useful background reading

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CONSERVATION OF GERMPLASM OF WILD SPECIES IN EUROPE

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Although a number of cooperative programmes for genetic resources exist between some European countries there is no overall plan or strategy that covers the whole of Europe or the European Community. Suggestions for a European Community Genetic Resources Programme have been made and an EC initiative is under consideration. A number of countries in East and West Europe already participate in the European Cooperative Programme for the Conservation and Exchange of Genetic Resources (ECP/GR), coordinated by IBPGR, but this is mainly concerned with a small number of major crops available in the participating countries.

The so-called 'formal sector' or institutional gene banks in Europe, such as those at Braunschweig and Gatersleben (Germany), Bari (Italy), INIA (Spain), and the Nordic Gene Bank, are primarily concerned with crop plants such as cereals and forages, although an increasing interest has been shown in recent years in the relatives of crops and other wild species. This concern with wild species will increase substantially as a result of their recent concern with biodiversity and the agreements made at the UNCED in Rio de Janeiro in June 1993, notably the Convention of Biological Diversity which may be ratified and come into force in 1993.

Wild species are the primary concern of many botanic garden and arboreta gene banks, notably that of the Royal Botanic Gardens, Kew at Wakehurst Place, and specialized collections such as the Cruciferae Germplasm Bank at ETSIA, Madrid. These, together with University and other special collections, constitute the 'informal sector'. The majority of botanic gardens hold germplasm as "living" material, either in their general collections or as special conservation collections, and data on these holdings are being centralized by the database of Botanic Gardens Conservation International (BGCI).

Groups of wild species of particular concern, in addition to crop relatives, are medicinal plants and culinary herbs, ornamental and landscape plants and those needed for habitat restoration or rehabilitation. Also, recovery programmes are being prepared for rare and endangered wild species and these often require the building up of of germplasm collections, for reintroductions or reinforcement of populations. Special attention is currently being focussed on the need for germplasm of forestry species.

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vhh/march/5/2/93.

TUESDAY, FEBRUARY 23rd

Wild genetic resources of cultivated plants in the Mediterranean Basin and the possible strategies for their *in situ* conservation

DANIEL ZOHARY

The wild relatives (wild progenitors) of cultivated plants are recognized today as a vital genetic resource for the future maintenance and improvement of agricultural crops. Plant breeders are increasingly concerned with these wild plants because their naturally growing populations frequently contain useful, untapped genes. Most important are genes conferring resistance to pests and pathogens which cripple crops. In numerous crops the disease and pest resistance sources that are present in the cultivated forms have been practically exhausted. For protection against attacks of new virulent races of parasites, breeders are increasingly turning to the wild relatives of the crops (the "wild gene pools").

While the economic importance of the wild progenitors of the crops is by now universally appreciated, there is yet much less concern about their survival. Many wild progenitors (such as the wild relatives of wheat, cabbage or grape-vine) occupy ecological niches which are being rapidly destroyed by man. The ever-increasing damage to their natural ecosystems is already causing considerable erosion of their genetic variation. Indeed, some have already joined the category of endangered species.

The Mediterranean Basin and the Near East constitute one of the world's richest centres for wild progenitors of cultivated plants. Yet very few serious attempts have been made by the various Mediterranean countries to assess their wild genetic resources of crop plants and to find out what should be done about them in terms of "in situ" conservation. There is an urgent need to answer the following questions:

- What is the inventory of the wild genetic resources in each country ?

This together with detailed information on the geographic distribution, ecological specificities and range of variation of each wild type - in each of the countries.

- Which of the wild progenitors in these countries require (or will soon be requiring) protection?
- What measures of "in situ" protection would be effective?
- What could be the contribution of the existing protected areas (nature reserves, national parks, etc.) in the various countries to conservation of these plants?
- What should be the role of the national authorities concerned with environment protection in the various Mediterranean countries in future "in situ" conservation of their native wild progenitors?

Finally, the background botanical and genetic information necessary for "in situ" conservation decision-making is yet largely unavailable in the case of the Mediterranean wild progenitors. This is in spite of the facts that (a) the methodologies for modern taxonomic revision and ecological assessment of the concerned plant groups are available, and top-rank botanists could be attracted to conduct the studies; (b) the Mediterranean Basin is (relative to other areas) well explored in terms of its vegetation cover, and is also accessible for field studies; (c) progress in molecular biology has recently added a whole battery of new critical tools for the assessment of genetic variation in natural populations (e.g. electrophoretically discernible protein markers; restriction fragment length DNA polymorphism).

**Germplasm collections. What we learned
and what we can learn from them.**

J.I. Cubero, ETSIAM, Córdoba, Spain

Germplasm collections became a priority in the last fifty years but they had already been formed and studied for at least two centuries. Obviously, different historical periods collected different vegetal materials and emphasized diverse scientific and practical objectives. Germplasm collections have produced many facts out of which valuable scientific theories have been proposed. Their "balance sheet" clearly being positive, there are however some negative points in their formation and handling which should be converted also in positive experience for the future.

First collections consciously maintained as such probably were those of medical plants kept in many monasteries during the Middle Ages. The empirical pharmacological knowledge obtained from them is out of the scope of the present paper. Monasteries also maintained garden plants whose number was increasing following East-West contacts (as Crusades were). The interest in keeping and studying these materials motivated the creation of the first botanical gardens, not surprisingly by universities (Padua being the first one in 1545). Private collections of tulips triggered the first recorded "plant collection fever" as well as the first intense plant breeding activity as early as in the XVI century in The Netherlands. Collections performed by of both private and public organizations were continuous since that time until now. Most, if not all, collected plants till the end of the XIX century were ornamentals. The increasing importance of the professional plant breeding motivated the first collections of varieties of agricultural interest. Extensive germplasm collections were performed during the first third of our Century, with H.V. Harlan and, especially, N.I. Vavilov as outstanding names in this field.

Among the many positive facts derived from germplasm collections worth of mentioning are: (1) the foundation of very precise taxonomic systems derived from the accurate description of varieties and forms of ornamental interest requested by gardeners from professional botanists in the first half of the XVIII century; (2) the interchange and *ex-situ* domestication of valuable materials probably since the end of the XVIII century; (3) the knowledge of phytogeographical areas around the world as a consequence of the precise description of collecting sites; (4) the always increasing scientific interest in describing the amazing amount of variation recorded in living collections, which lead to the establishment of the modern Plant Systematics;

(5) countless varieties of commercial use in all fields, both purposely and unconsciously (through accidental hybridizations in maintained living collections), and new methods to obtain them (wide and narrow crosses, for example) which were transferred to the common scientific practice (not to be forgot: Mendel selected his pea lines out of a living commercial collection maintained in his monastery); (6) experimental knowledge on population dynamics, as the well known Vavilov's laws of "parallel variation" and "migration of recessives" in cultivated plants; (7) a deep insight into the origin and evolution of cultivated plants as well as on the host-parasite co-evolution and on the causes of genetic erosion; (8) a strong-effort on setting up priorities in germplasm conservation and on the best technical ways to implement them, and a long et cetera.

There are also some negative aspects on germplasm collections, or rather on germplasm *amassing*; among others; (1) losses produced when, for example, collections are tried to be kept in places without the required human and material equipment for such a delicate task, a problem already detected in the XIX century and not yet completely solved; (2) development, especially at the national level, of a "stamp collector syndrome", i.e., collecting because nowadays it is a fashionable task, having the required infrastructure but not having clear ideas on why and how to use or to study the material collected; (3) spoliation of natural environment on behalf of commercial and/or scientific interests; (4) jealousy at many levels (personal, professional, national and supranational) which hinders a correct flow of information.

While still learning in a positive way from plant collections, our best contribution for the next future could certainly be to eliminate the negative aspects that preclude the right use of actual germplasm collections.

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Transfer of Alien Genetic Variation in Plant Breeding

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Conventional plant breeding programs have been highly successful in all crops producing a steady stream of new improved cultivars. Thus these are elite genetic entities that combine the currently best available combinations of yield with tolerance to biotic and abiotic stresses and value-added traits. One of the immediate deleterious effects of a wide cross is the dilution of this elite germplasm which then will require numerous backcross generations to restore it.

Considering the immediate deleterious effects of wide crosses they are undertaken to introduce traits that are currently lacking in the primary gene pools of crop plants. Such traits include resistance/tolerance in wheat to fungal head blight caused by *Fusarium*, to Barley Yellow Dwarf Virus and to the wheat curled mite. Urgent requirements in barley include resistance/tolerance to the leaf spotting root rot complex caused by *Helminthosporium* and resistance to a new race of stem rust. In addition, cereal crops in temperate climatic zones can all benefit from additional genetic variability for such abiotic stresses as cold, heat drought and salinity.

There are several hundred species of wild relatives of crop plants in the tribe Triticeae that carry most or all of the above mentioned desirable traits but their transfer requires meticulous manipulations. The initial production of hybrids is accomplished by hormonal treatments of the maternal parents and embryo rescue on steadily improving media. Hybrids or derived amphiploids are repeatedly backcrossed to ideally produce a complete series of

alien chromosome addition lines. Recombination is then induced between the critical chromosome addition lines and a crop plant chromosome. Recombination can be induced by callus culture, use of mutants of meiotic pairing control genes or suppressors of such genes.

Existing RFLP maps of various crops and species specific probes employing in situ hybridization techniques can now effectively be used to detect the size and site of interaction of alien chromosome segments. Additional manipulations are often required to reduce the size of such segments while retaining the traits in question. A simplified version of the RFLP technique, the RAPD-PCR method employing random or specific primers has been effectively employed to provide tags for integrated disease resistance genes. Such tags should prove to be amenable to improving the efficiency of selection for plant breeding purposes. Examples of the above manipulations from ongoing programs will be illustrated and discussed.

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PLANT CONSERVATION: THE CASE OF WILD SPECIES

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A number of case-histories is presented for as many Spanish wild species which are either rare or directly threatened. They exemplify the kinds of threats and the different situations that can be found while an adequate conservation policy is being planned. Though wild plants should be preferently conserved "in situ" the difficulties in the simultaneous consideration of a high number of species in an endemic-rich country such as Spain, do advocate for the parallel use of "ex situ" techniques. For orthodox species, seed banks provide an emergency conservation method to avoid possible extinctions. Two cases of plant species saved from extinction by "ex situ" techniques are presented. Seed banks can also contribute heavily to stimulate botanic research by providing rare plant material otherwise very difficult to obtain. With respect to seed banking procedures, it is felt that they should not blindly follow what is being done in larger crop seed banks; as the number of samples tend to be smaller and of smaller size for wild species, the techniques used are in most cases more precise and reliable.

WEDNESDAY, FEBRUARY 24th

DNA conservation and the DNA Bank-Network
Dr. Robert P. Adams, Baylor University, USA

Genetic transfers from insects, bacteria, viruses, animals, and plants to unrelated organisms indicate that we must now view the world's genetic resources (genes, DNA) from a horizontal perspective in which gene transfers will cut across species, genera and family boundaries. The world's biota should now be considered a **horizontal gene pool**.

Previously, we have utilized only vertical gene pools (i.e., breeding with ancestral or derived taxa that are closely related in order to make fertile or semi-fertile crosses. The development of pharmaceutical farming, bioreactors and even insect resistance in our field crops will now utilize genes from distantly related taxa (i.e., the **horizontal gene pool**).

The recent advances in technology for the extraction and immobilization of DNA, coupled with the prospect of the loss of significant plant genetic resources throughout the world, has led to the establishment of DNA Bank-Net. DNA Bank-Net is an association of institutions focused on preserving DNA as well as in vitro cryopreservation of plant cells. There are over forty institutions, representing 25 nations and every continent, that have expressed interest in DNA Bank-Net.

Topics:

I. Structure and Operation of DNA BANK-NET

A. Working (DNA dispensing) nodes

- a. Collection of plant material by taxonomists.
- b. DNA extraction by molecular biologists or trained staff.
- c. Long-term preservation of DNA-rich materials and/or extracted DNA in liquid nitrogen.
- d. DNA analysis/gene replication by molecular biologists or trained staff.
- e. Distribution of DNA (genes, gene segments, oligonucleotides, etc.).

B. Reserve (base) nodes:

- a. Long term DNA preservation in liquid nitrogen and monitoring of potential DNA degradation.
- b. Act as genetic reserve buffer for working nodes.
- c. Replenishment of DNA if a working node experiences the catastrophic loss of storage parameters and DNA.

II. General Requirements for Nodes in the DNA BANK-NET

III. Plant specimens collectors - an underutilized resource

IV. Interim Field Preservation of Specimens

V. Future Directions

- A. in vitro amplification
- B. immobilized DNA

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IN VITRO CONSERVATION OF GENETIC RESOURCES

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The maintenance *in vitro* of plant material has an important role to play as an alternative to field genebanks in conserving clonally propagated species (eg. potato, cassava, yam, taro, cocoyam, banana, garlic), and those species which produce recalcitrant seeds (eg. cacao, mango, coconut, avocado, hops). Increasingly, *in vitro* conservation is even required for seed crops such as barley, rice and maize, where because the possibility for transformation is governed by genotype, there is a need to conserve competent cell cultures for continued experimentation.

Various techniques now exist for *in vitro* conservation including slow growth storage and cryopreservation, the latter being the most promising for long-term conservation of germplasm. While slow growth storage is dependent upon the ability to culture shoots under conditions which will reduce the growth rate to a minimum, cryopreservation depends upon the manipulation of meristems, callus, cell cultures or somatic embryos. Success may depend upon preconditioning, cryoprotection, rate of freezing, and the use of vitrification and desiccation strategies before storage.

In vitro storage of germplasm of potato, cassava and banana is now underway. Research continues to find ways of conserving mango, coconut and *Xanthosoma*. *In vitro* techniques for collecting germplasm are well advanced and have been successfully applied to cotton, cassava, coconut and citrus fruits.

Genetic stability of germplasm stored *in vitro* is an important consideration. Somaclonal variation can be induced through *in vitro* culture, and may occur at very high levels. For instance, in bananas and plantains up to 60% of *in vitro* progeny can be offtypes. Despite this, *in vitro* techniques are still preferred for propagation and maintenance of germplasm. Because of the significance that *in vitro* techniques have in *Musa* germplasm conservation and propagation, a case study of bananas and plantains is worthy of deeper consideration.

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CONSERVATION OF GENETIC RESOURCES OF WOODY SPECIES WITH RECALCITRANT SEEDS: PROBLEMS AND PERSPECTIVES

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Seeds of many woody species, including most fruit trees, loose viability very quickly after desiccation and freezing, and from the practical point of view are recalcitrant. The consequence is that conservation of genetic resources of these species is done as collections of living trees growing in the field (field genebanks).

There are many problems associated with the maintenance of field genebanks. There is a high risk of losing plants due to climatic hazards (e.g. freezes, strong winds, flooding) and to diseases produced by fungus, bacteria, virus and virus-like pathogens. Plants of fruit trees are highly heterocigoticus and they are vegetatively propagated to maintain the genotypes. In consequence, the exchange of germplasm is generally done as budwood, and this has a very high risk of dissemination of pests and diseases. The presence of diseases may also change the morphological characters of plants. In addition, there is a lack of biochemical methods for characterization of closely related genotypes, that has to be done following time consuming morphological methods. This facilitates the duplication of genotypes, that are known by local names in different regions. Finally, the maintenance of tree collections is very expensive, due to high labor and land surface needed.

There are several approaches to solve the problems mentioned above. The development of efficient cryoconservation methods could be the best solution, although at present time this technology has very little practical application. Methods to grow plants in containers in insect-proof screenhouses for long periods of time will reduce the space needed for conservation and it will avoid infection with diseases. Additional research is needed to develop quick and simple methods for biochemical identification of closely related germplasm, for disease elimination and for safe exchange of germplasm. The conservation of only pathogen-free plants is a high priority for many species, and this is already being done for some of them. The inclusion of healthy accessions of commercial interest in the germplasm banks is an excellent means to obtain financial support to cover the high expenses of maintenance.

All these problems and perspectives are discussed with the case study of citrus, which are maintained in a large collection of germplasm composed only of pathogen-free plants at the Instituto Valenciano de Investigaciones Agrarias.

Methods of Identifying Useful Germplasm

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Empirical data from three intensively studied crop groups will be examined to identify genetic changes that occurred as wild species were converted into land-races and then into elite cultivars. First to be examined are changes that occurred over generations in the frequencies of discretely recognizable alleles of Mendelian loci that govern various morphological, disease resistance, allozyme and restriction fragment variants. The main findings are that the frequencies of such alleles are highly correlated with adaptedness, productivity and product quality; evidently natural and/or man-guided selection for improvement in these attributes caused the frequencies of some of these alleles to reach high levels and the frequencies of most such alleles to reach low levels over generations. Fortunately the frequencies of discretely recognizable alleles can be determined quickly, with great precision, and relatively inexpensively by simple counts of numbers of different alleles in various sources of exotic germplasm (e.g. accessions in germplasm banks, genetically enhanced populations), as well as in current local breeding stocks and cultivars. Rapidly growing arrays of discretely recognizable variants, including restriction fragment variants, thus appear to offer outstanding opportunities for identifying promising alleles for introgression into local breeding stocks. If an allele is frequent in some area but not present in other areas, prudent genetic resource management calls for introducing such alleles into the areas where they are not present. If an allele is rare everywhere it is unlikely useful anywhere and cost-effective germplasm managers will direct their efforts elsewhere.

The task of managing germplasm at the genotypic level is much more difficult than at the allelic level. This is because having superior alleles in breeding stocks is not enough - superior alleles must be assembled into synergistic multilocus combinations that give wide adaptedness and high performance over the range of fluctuations which occur in local environments. This is a substantial complication because the numbers of possible genotypes increase exponentially with increasing numbers of loci and increasing numbers of alleles per locus. The consequences are that large numbers of cycles of segregations and recombination, carried out in large populations, are required merely to produce the most useful multilocus genotypes and further, that laborious and expensive testing under agricultural conditions is required to determine the real value in any given environment of novel genotypes.

The concept which emerges is that preservation of biodiversity, as well as the utilization of genetic resources are evolutionary processes and that understanding of the underlying evolutionary mechanisms responsible for the genetic changes that have occurred over generations provides the most certain guide for development of effective management strategies for the future.

Plant Genetic Resources Evaluation and Gene Transfer

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A conceptual framework for evaluation of PGR and gene transfer will be presented and illustrated by a few examples. First, the goals of the evaluation/breeder need to be clarified. For example, there may be a search for a specific critical trait or, alternatively, general evaluation to see what is present in the targeted species with the hope that some previously unknown characters can be uncovered. The end-use goals must also be articulated because a genetic resource could be identified and multiplied for direct use in agriculture or the genetic resource may supply only a single gene to a modern cultivar. Second, the type of genetic resources to be evaluated may be landraces, progenitor species, or wild relatives and these may require different evaluation/gene transfer processes. Third, an assessment must be made about the conservation status of the targeted species with respect to in situ populations and ex situ collections. If there is insufficient conserved genetic resources, the evaluations of existing ex situ collections may be futile. Fourth, the documentation/characterization status of the materials to be evaluated should be reviewed, especially with reference to eco- geographic history because "environment foretells certain characteristics about the genotype". Fifth, sampling strategies for evaluation of both in situ and ex situ genetic resources must be sufficient to ensure the capture of unique, and perhaps rare, traits. In situ evaluations may be useful, but somewhat difficult, more so for wild species than landraces. For ex situ collections of reasonably large size it will usually be necessary to stratify the collection so that certain proportions of the collection may be evaluated sequentially. This is because of physical limitations and also because the goal may be to evaluate the collection only to the extent necessary to discover a desired trait. Sixth, evaluation methods must consider both the measure by which a trait may be evaluated or observed and the environmental design to ensure accurate phenotypic classifications. These considerations include stress vs. optimum environments and replication or other experimental design aspects. Seventh, and this is extremely important, is genetic validation to prove that an observed trait is actually genetically controlled and if so, whether it is oligo- or multigenic. This requires progeny-testing and controlled hybridization studies. Eighth, gene transfer strategies include classical plant breeding methods such as mass selection and backcrossing or parasexual methods of gene isolation and transformation. Generalized conversion of genetic resources such as to photoperiod insensitivity, may be useful or necessary in order to carry out proper evaluations or to use the genetic resources in breeding programs.

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Chromosome engineering and gene transfer from wild to cultivated cereals

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The wild *Triticeae* species contain a wealth of genetical variation which can be utilized for the improvement of the cultivated species - bread wheat, durum wheat, barley, oats and rye. Of particular interest are genes for disease and pest resistance, tolerance to abiotic stresses such as salt, waterlogging, drought and heat, and quality attributes such as grain protein content. The first step in a gene transfer programme is the proper characterization of this variation and the development of suitable tests for its detection and exploitation.

During the evolution of the *Triticeae* the basic primeval genome of seven chromosomes has evolved and changed following speciation and allopolyploidization but sufficient homoeology exists between the cultivated and wild forms for gene transfer to be possible by sexual crossing and chromosome engineering. Using artificial hybridization and embryo rescue technologies enables incompatibility barriers to be overcome, and interspecific and intergeneric hybrids can be created and developed into viable seedlings and plants. From these plants, stable amphiploids can be created by chromosome doubling, or the hybrids can be utilized directly for gene transfer by backcrossing to the cultivated species of interest.

Alien variation can be incorporated into cultivated species at three levels - first at the level of the whole genome, as in triticales (rye genome); secondly at the level of the individual chromosome or chromosome arm, as for example the 1BL/1RS chromosome in wheat (rye 1RS); and thirdly at the level of small chromosome segments containing individual genes, such as *Sr24* for resistance to stem rust on wheat chromosome 3D (3Ag⁺ segment from *Thinopyrum elongatum*). To exploit whole genomes requires the production of many different amphiploids, intercrossing these and selecting for cytological stability, fertility and good agronomic performance. Examples of such an approach are triticales, Tritordeum and Tritipyrum.

The incorporation of only parts of the genome of an alien species requires chromosome engineering techniques and can be directed or at random. "Shotgun" introductions, where unknown, random, segments of the alien genome are introduced, are achieved by backcrossing either the amphiploid or hybrid to the cultivated species and selecting for the trait of interest in the progeny. However, such plants often suffer problems of cytological instability and poor agronomic performance because of an unbalanced genome, and a directed approach is to be preferred. This involves first developing alien addition and substitution lines and then using these to derive progenies containing homoeologous chromosome transfers either by using ionizing irradiation or by manipulating the mechanisms of chromosome pairing.

Chromosome engineering also requires efficient marker systems to enable recognition of, and selection for, the alien chromatin in the cultivated species genome. Initially, morphological markers were used but these have now given way to biochemical

markers (isozymes, storage proteins), molecular markers (RFLPs, RAPDs) and *in situ* hybridization techniques (specific probe *in situ*, genomic *in situ*). These now enable the alien segments to be recognized and followed as well as enabling the whole genome composition and genetical stability of derived progenies to be assessed.

There are now many notable successes from chromosome manipulation and engineering in the small-grained cereals, particularly in bread wheat and oats. However, the other cultivated species, for example barley, are gradually becoming amenable to such manipulations. It is expected that wild *Triticeae* species will continue to have an increasingly important role in supplying genetic variation to combat an ever changing spectrum of diseases and pests, and hostile environmental conditions, to which the cultivated forms are being subjected.

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POSTERS

ISOZYME CHARACTERIZATION OF THE WILD BEET *BETA COROLLIFLORA*
ZOSS, AND ITS HYBRIDS WITH *BETA VULGARIS* L.

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The wild beet *Beta corolliflora* Zoss. belongs to the section *Corollinae* of the genus *Beta*. It is tetraploid ($2n=4x=36$) and presumably an autotetraploid.

B. corolliflora has been reported to contain genes for resistance to curly top virus (Beta Virus 1), mosaic virus (Beta Mosaic Virus 2) and *Polymyxa betae*, the carrier of the *Rhizomania* virus.

There are very few studies on *B. corolliflora*. With the use of isozymes, we try to look into the genetic composition of this plant as well as its hybrids with *Beta vulgaris* for breeding and taxonomy purposes.

MULTILOCUS GENETIC STRUCTURE IN POPULATIONS OF *Avena barbata* IN NORTHERN SPAIN

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It has been reported that genetic variability in highly inbred species, such as wild and cultivated oats and barley, is structured in multilocus associations, whose distributions are related to environmental parameters. This observation is of crucial importance in the strategy used in conservation projects as they presumably represent adaptations to significant local variation in the environment, and therefore they may be of special interest for plant breeders.

Our previous works carried out in Spanish populations of *Avena barbata* showed a high degree of differentiation among populations, in such way that only one multilocus association was present in several populations with a relatively high frequency. This association has been named North Association, and the results suggested that natural selection could be responsible of its maintenance in populations.

In order to study this hypothesis, we sampled about 100 plants of each of 39 populations growing in North Spain. Using starch gel electrophoresis, 14 isozymatic loci have been checked individually, and a discrete log-linear analysis has been carried out to analyze to see whether the North Association is significantly present in the zone. The observed geographical distribution supports the idea that natural selection is a main factor in the structuration of the variability, and in the maintenance of this multilocus association in the area sampled.

Application of RAPD markers for apricot characterization

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Abstract:

Dr. Maxine Thompson in 1988 collected populations of Pakistani apricot (*Prunus armeniaca*, L.) which were distributed by the National Clonal Germplasm Repository at Davis. This collection comes from the central Asian center of origin as defined Vavilov and Kostima. The collection includes, probably, important adaptation characters and may be invaluable as a future source of desirable genes.

A number of 10 base primers were screened to identify RAPD polymorphisms of these semi-wild apricot genotypes. Different genotypes collected at 4 locations in Pakistan were analyzed. DNA from leaf tissue were isolated and tested against 15 primers.

Seven primers, O-05, O-06, O-07, O-08, O-10, and O-13, were identified that produced consistent results with relatively few (though scorable) and consistent bands.

DNA was isolated using the CTAB method and the effect of additional CsCl centrifugation isolation was tested. No significant differences were found. In addition, reaction conditions were tested to ensure consistent results. RAPD polymorphism was observed in these genotypes indicating that this technology is a powerful tool for apricot characterization.

Abbreviations: RAPD: Random amplified polymorphism DNA; CTAB: hexadecyltrimethylammonium bromide; CsCl: Cesium Chloride.

Key words: Apricot, Molecular markers, Polymerase Chain Reaction, Polymorphism, Primers, *Prunus*.

Isozymes for grapevine characterization

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Abstract

Isozyme electrophoresis has been widely used for characterization of plant material at the germplasm banks. One of the objectives of this experience was to establish a standard methodology for characterization of grapevine cultivars at the *Vitis* germplasm banks in Spain.

Isozymes in extracts of woody stems from 10 grapevines (*Vitis vinifera* L.) were analyzed. Separation was carried out in 10% polyacrylamide gels with specific staining for the following systems: Peroxidase (PER), Esterase (EST), Catechol oxidase (CO), Glutamate oxalacetate transaminase (GOT), Cytochrome oxidase (CIT) and Acid phosphatase (AcP).

Results of the analysis yielded specific profiles for most of the studied varieties, being able to distinguish them. CO resulted the most discriminant system, producing 7 different profiles for the 8 studied varieties. 'Tinto Fino' and 'Tempranillo' clone 43, shared the same profile for all the isozyme systems. 'Airen' and 'Cabernet Sauvignon' showed clearly distinct profiles for the GOT. Also 'Bobal' showed specific profiles for PER and CIT, that were quite different than for the rest of the varieties. Interclonal differences were not observed for any of the studied isozyme systems.

At the present time, other techniques such as starch gel electrophoresis and RAPD markers, are under study in this laboratory in order to characterize grape varieties.

Key words: Characterization, Grape, Isozymes, *Vitis*.

NEW STRATEGIES FOR EX SITU CONSERVATION OF PLANT GENETIC RESOURCES: II. CRYOPRESERVATION

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Preservation at very low temperatures (-196°C , liquid nitrogen) is becoming an alternative when traditional storage systems (seed banks, live collections, *in vitro* conservation ...) are not feasible. Cryopreservation can improve the storage conditions or the cost effectiveness with respect to those other methods. For instance, the use of liquid nitrogen (LN) as a storage medium would be preferred over mechanical refrigeration because of its reliability and easy use, as it only requires the addition of LN to the reservoir on a periodic basis. Besides, at such low temperatures (-196°C), metabolism comes to a halt, with no changes in the genetic characteristics.

The germination capability of seeds with several moisture contents was tested after storage in LN for different periods of time in several wild and crop species. In most of them no significant differences were found between control and LN preserved seeds.

The viability of embryonic axes of two woody species with seed storage problems (*Quercus faginea* Lam. and *Coryllus avellana* L.) was studied after their desiccation and direct immersion in LN. Desiccation improved germination percentages of frozen embryonic axes.

Axillary buds from micropropagated *Centaurea rigualii* Esteve shoots survived freezing at -196°C when previously treated with high concentrations of cryoprotectants (vitrification). A preculture period of two days at low temperature on media containing some cryoprotectants increased the surviving rate. Protocols are still under study to improve the percentage of buds elongating after freezing.

Meristems of *Populus nigra* L. encapsulated in alginate beads were dehydrated and frozen in LN. Their development was studied after culturing on semi-solid medium.

The germination of pollen of *Erodium paularense* Fdez.-Glez. & Izco was studied before and after its storage in LN.

These experiences show that it is possible to develop appropriate cryopreservation techniques for each type of plant material. The importance of preserving genetic material demands that the best and safest possible storage techniques are employed. LN storage appears to be both practical and desirable for long term preservation of several plant propagules.

Conservation genetics of an endangered plant species, *Hippocrepis valentina*.

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One of the main tasks faced nowadays in Biology refers to the preservation of its very same object of study, living organisms. The conservation of biodiversity requires a global, multidisciplinary approach. There are several contributions to this goal amenable from Genetics. This work presents the results of the analysis of isozymic variability of several natural populations of a plant species, *Hippocrepis valentina*, endemic of the east Mediterranean coast of Spain and currently with status of endangered species.

Our results, obtained by starch gel electrophoresis of 15 enzyme systems, show normal levels of variability for species with a similar biology, with most variation occurring between populations, and with an excess of heterozygotes for several genes within populations.

The comparison with the patterns of genetic variability of two closely related species, *H. balearica* and *H. grossi*, confirms the taxonomic status of *H. valentina* as a proper species, independent of *H. balearica* as previous hypothesis had suggested.

The information obtained in this study has direct applications for the conservation of this species, both in the design of an adequate policy for the protection of natural populations (*in situ* conservation) as well as in providing the necessary bases for the collection and keeping of seed banks (*ex situ* conservation), where the variability currently found can be preserved for the future.

Grass pea (Lathyrus sativus L.): Use of Molecular Markers and Future Implications in the Eradication of Lathyrism.

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Grass pea (*Lathyrus sativus* L.), is a drought resistant, protein rich food legume extensively cultivated in Central Asia, East Africa and also some relict regions of the Mediterranean Area.

Excessive consumption by human and animals of Grass pea seeds, is reported to cause a paralytic disease "*Lathyrism*" because the seeds contain a neurotoxin, β -N-Oxalyl-amino-L alanine (BOAA).

In the last twenty years, several breeding programs have been carried out to decrease the level of this neurotoxic compound. In this way the availability of molecular markers, particularly isozymes, and restriction fragment length polymorphism (RFLPs), will greatly facilitate selection programs, to investigate systematic problems or to measure levels of variation within and among populations. Furthermore a knowledge of the distribution of the genetic variation is of primary importance for the conservation of genetic diversity of plant species.

We have carried out the isoenzymatic analysis of different accessions of Grass pea covering the area of distribution, including both primitive landrace forms as well as more advanced varieties. Our results suggest that the primary gene pool of *L. sativus* is extensive.

Molecular markers also provide new information about gene map of different genes. We have carried out an isoenzymatic linkage analysis in Grass pea, suggesting that some linkage groups are conserved among different species of Legumes: Bean, Chickpea, Lentil and Pea, and including Grass pea.

NEW STRATEGIES FOR EX SITU CONSERVATION OF PLANT GENETIC RESOURCES. I. MICROPROPAGATION AND LOW-GROWTH IN VITRO STORAGE.

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In the last two decades, the success of conventional seed banking techniques has been responsible for the large development of *ex situ* conservation programs throughout the world. However, the existence of recalcitrant seeds as well as other factors such as economy, term of storage and passive-safety require the development of new strategies for *ex situ* conservation that give an alternative way of storage to every plant species and improve the above-mentioned factors.

Our department became involved in *ex situ* conservation in 1964, when a seedbank was established. Throughout the years, several seed collections have been created, the most important being the collections of wild crucifers and Spanish endemic species.

With the purpose of exploring new ways of *ex situ* conservation, a micropropagation unit was created in 1988 and a cryopreservation unit in 1991. Our search for new ways of *ex situ* conservation, has taken two directions: the development of new culture and storage techniques and the consideration of propagules other than seed.

In vitro culture techniques can be an alternative to seed banks in the case of recalcitrant seed species, species for which the flowering stage is not known, with low fertility or, low seed or pollen production, highly heterozygous cultivated clones, perennials with long life-cycles that do not set seed until a considerable number of years, and highly restricted species where the simple gathering of seeds in the natural population may affect the survival of the population.

For the last four years we have successfully micropropagated and transferred to *ex situ* conditions in the greenhouse, *Centaurium rigualii* Esteve, *Coronopus navasii* Pau, *Lavatera oblongifolia* Boiss., *Limonium calaminare* Pignatti ex Pign., *L. catalunicum* (Willk. et Costa) Pign., *L. dichotomum* (Cav.) O. Kuntze, *L. duforei* (Girard) O. Kuntze, *L. estevei* Fdez. Casas and *L. giberitii* (Sennen) Sennen (endemic plant species from the Iberian Peninsula, most of them threatened).

Erodium paularense Fdez.-Glez. & Izco and *Centaurea lainzii* Fdez. Casas, also threatened, are at different stages of micropropagation, at the present time.

Low-growth *in vitro* storage experiments have been carried out with all the species that have been micropropagated. For each species, different temperature, light, growth regulators and sucrose conditions were used. The best general storage conditions were reached at 5°C in a MS medium alone or with 1 mg/l BAP and 0.1 mg/l NAA. Under these conditions, over 60% survival was obtained in all species for periods of at least six months and up to two years, depending on the species.

An often mentioned problem of *in vitro* culture techniques is the possibility of obtaining somaclonal variants among regenerated plantlets. The choice of an adequate explant, cycle of micropropagation and, types and concentrations of growth regulators may contribute to reducing the appearance of somaclonal variants but will not completely eliminate the chances of getting them. On the other hand, the scarceness of starting plant material, especially with threatened plant species, further difficults the process of micropropagation leaving little margin for previous considerations.

Therefore, we have considered it to be of interest to assay different methods of detection of somaclonal variants among regenerants. Thus, we have carried out morphometric studies with a computer assisted image analysis system, isozyme studies, citological observations and DNA analysis through PCR/RAPD in several of the micropropagated species. The last two techniques are, in theory, able to give more conclusive results since they get closer to the gene than the other two. However, it must be observed that all of them have a limited span (do not check the integrity of the whole genome). The choice of one technique or another, or none, will depend on each particular situation or species, lab equipment, economy and purpose of conservation.



**Random Amplified Polymorphic DNA markers as an useful tool for
Rose cultivar identification.**

T Millán, AM Torres and JI Cubero

It has become increasingly important to develop highly reliable and discriminatory methods for cultivar identification. Plant breeders, nursery industry and plant growers, need sensitive tools to differentiate and identify cultivars for the purposes of plant patent laws. Methods have been developed over the past twenty years that allow the detection of differences in DNA. In comparison with isozymes, these polymorphisms have numerous advantages: they are independent of environmental conditions, their expression is identical with whatever stage or tissue of the plant it may be analyzed and the number of scorable loci is unlimited.

Five rose cultivars were analyzed using Random Amplified Polymorphic DNA (RAPD) markers. Only with eight primers all cultivars could be distinguished by comparing differences in their DNA banding pattern. Each cultivar yields a single pattern of DNA bands. Since limitless number of primers can be assayed and several DNA bands can be differentiated for each one, the number of possible combinations is infinite. In the present work, differences among cultivars were obvious and expressed consistently in most of the primers. Although additional work is needed to refine and expand the results obtained from this initial study, our results confirm that the RAPD technique is to be useful to characterize rose cultivars.

GENETIC IMPLICATIONS OF THE BASIC MULTIPLICATION OF GENETIC RESOURCES OF *Centrosema* spp.

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The family **Leguminosae** is one of the most important botanic groups, having a large number of species, many of them of economic interest and probably the greatest genetic variability.

Centrosema is a member of the diverse **Phaseolae**, one of the largest leguminous tribes. It is native from Brazil and Central America, having about 35 described species, most of which originated in the Central West Region of Brazil. These species have great potential as forage, being indicated as an alternative source of protein during the dry season.

Different accessions belonging to three species (*C. acutifolium*, *C. brasilianum* and *C. pubescens*), were collected in Brazil and multiplied in two sites, at EMBRAPA in Campo Grande, MS, Brazil and at CIAT, in Cali, Colombia. This material was submitted to analysis by starch gel electrophoresis. The isoenzymatic systems analysed were Glutamate Oxalacetate Transaminase (GOT), Phosphoglucose Isomerase (PGI), Phosphoglucose Mutase (PGM), Malate Dehidrogenase (MDH), 6-Phosphogluconate Dehidrogenase (6-PGD), and Isocitric Dehidrogenase (IDH).

We have observed important differences in genetic variability among accessions collected in the same area and multiplied at the two sites.

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COLLECTION AND EVALUATION OF WILD POPULATIONS OF PERENNIAL RYEGRASS FROM GALICIA (NORTH WEST OF SPAIN)

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In order to set up a programme on perennial ryegrass improvement at the Agronomic Station of Mabegondo (Galicia), an intensive collection of wild populations has been made in Galicia during the summer 1985 and 1986. In 1990 other populations were collected in Asturias, Cantabria and Pais Vasco (North of Spain). 47 of these populations have been evaluated for forage traits only at Mabegondo Station and the others being evaluated at 5 locations (2 in Spain and 3 in France). A hierarchical ascendant clustering on different agronomic traits leads to a classification into 4 clusters of populations.

Multilocal evaluation (1 location in Spain and 2 in France) of 21 out of 47 populations showed many genotype x environment interactions for forage traits such as susceptibilities and vigours and were taken into account through multivariate analysis.

A hierarchical ascendant method based on standardized euclidean distances leads to a classification into 6 clusters. Some clusters have a narrow geographic distribution and show specific regional adaptation. Two clusters show a particular profile in French and North West Spanish conditions.

Wild populations showed quite a wide range of variation for agronomic traits compared to the sample of cultivars evaluated.

Moreover the same appears to be situation for isozyme markers: preliminary results from 6 of the described populations (about 100 plants each) and 7 polymorphic loci (PGI, ACP, EST, PGM, SHDH, GOT, and PGD) indicate average heterozygosity of 0.395, mean number of alleles per polymorphic locus of 3.16 and average Nei's distance for every pairs of populations of 0.057.

A core collection has been set up by sampling populations with each out of 4 clusters, for multiplication and distribution on request. Seed samples and passport data of these and other populations were send to Genetic Resources of Perennial Ryegrass Unit of Aberystwyth (UK, Wales, I.Thomas).

Two broad base populations have also been built by intercrossing plants selected from two of the clusters. These base populations will be studied in order to know their genetic parameters (agronomic traits) and their genetic drift (isozyme markers).

KEY-WORDS: *Lolium perenne* L., Genetic variability, Multivariate analysis, Isozymes, Core collection

USE OF LOCAL GERMPLASM OF MAIZE TO DEVELOP VARIETIES ADAPTED TO SPECIFIC ENVIRONMENTAL CONDITIONS

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A program was started in 1974 at the Misión Biológica de Galicia with two objectives. First, to make a collection of maize (*Zea mays* L.) landraces from all over Spain with an emphasis on Galicia, the northwestern corner of the Iberian Peninsula. Second, to use those landraces to develop varieties adapted to the specific environmental conditions of Galicia, characterized by a humid climate, with some drought during the summer and cool, rainy springs.

At present 121 composites are maintained. Seventy-five of them are from Galicia and the remaining from other areas of Spain. These composites are kept in cold storage. To assure continuously a good quality seed, a composite is sown and multiplied by hand when its germinating power falls below 50 percent.

For the second objective two different systems were implemented. The first system is a classical pedigree selection scheme of developing first- and second-cycle inbreds from the composites. The second system is a reciprocal selection program along the lines of the comprehensive breeding system of Eberhart et al. [Züchter 37:169-174 (1967)].

Several inbreds have been obtained that show from average to good combining ability for yield. All of them are much better than standard Corn Belt lines for early vigor, a trait of paramount importance in the agriculture of the northern areas of Spain.

Ordás [Crop Sci. 31:931-935 (1991)] has recently reported the first results of the reciprocal selection program. In 1993, after three cycles of intrapopulation selection, a scheme of interpopulation selection will start following Hallauer's system [Egypt. J. Genet. Cytol. 2:84-101 (1973)] for reciprocal full-sib selection with non-prolific material.

EVALUATION OF PORTUGUESE *DACTYLIS GLOMERATA* L. GERMPLASM*

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Ecotypes have evolved through plant adaptation to specific edafo-climatic conditions such as, drought and water saturation, heat and cold, soil metal toxicities, pests, diseases, etc.

Genetic erosion due to mankind practices take to loss of important genes for plant breeding purposes. Therefore recolt, preservation and evaluation of germplasm is, nowadays, a major goal.

40 Portuguese ecotypes of cocksfoot (*Dactylis glomerata* L.), from five different regions of Portugal, were evaluated at Vila Real, in a spaced plant trial for 19 parameters according to the IBPGR descriptors. Two cultivar from Spain, Italy, Netherlands and New Zealand were used as controls.

A Main Component Analysis was used for an integrated study. It was observed a great variability within ecotypes and cultivars.

However, although overlaps between ecotypes of different regions occurs, it was possible to observe a general distribution according to the regions from where they were collected.

Cocksfoot ecotypes from Minho (Northern shore) were characterized by the parameter axis related to yield (growth) and plant vigour such as, length and width of flag leaf and width of plant at ear emergence, number of inflorescences, height at ear emergence and height in Spring. On an opposite position, ecotypes from Trás-os-Montes, Alentejo and Beira Interior (Northern, Centre e Southern inner region, respectively) were located. For the very same parameters these ecotypes presented lower values than Minho ecotypes.

The Beira Litoral (Centre shore) ecotypes had an intermediate distribution more close to the Minho ecotypes.

New Zealand and Netherlands cultivars form a small group. Italian cultivars had the same general pattern of Trás-os-Montes, Alentejo and Beira Interior ecotypes. The two Spanish varieties and one ecotype of Beira Interior differ from the other populations and are characterized by the lowest values for growth and plant vigour.

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Distribution of *Aegilops* species and wild *Triticum* species in worldwide germplasm collections

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There has been an increasing interest in the use and study of wild wheat relatives for incorporating useful genetic traits into breeding material. With respect to the wild relatives of wheat, namely wild *Triticum* species and *Aegilops* species, a large number of accessions have been collected over a wide geographical area and the resulting collections have been dispersed and stored in genebanks throughout the world. In order to determine evaluate the completeness of previous collections, target future collection missions more accurately, determine priority areas for collection, map the ecogeographical distribution of each species, examine the extent of duplication between institutes and improve utilization of the material, IBPGR (International Board for Plant Genetic Resources) in collaboration with ICARDA (International Centre for Agricultural Research in Dry Areas) compiled a centralised database containing ecogeographical passport data on wild wheat relative germplasm accessions and herbarium specimens held in 50 germplasm collections. To compile the data 111 institutes and collectors were contacted for information regarding their germplasm and herbarium collections. The germplasm database (currently housed in the Genetic Resources Unit of ICARDA and duplicated at IBPGR Headquarters, Italy) holds information on 20,000 accessions originating from 33 countries, 5000 of which were identified as duplicates, representing 14,872 unique accessions and gives a fairly comprehensive view of the existing status of the worldwide collection. The germplasm database in its present form contains 59 fields with information on species identification, collecting organisation, site locality, site characteristics and institutes holding each accession. The herbarium database contains data on over 2000 accessions. The herbarium database has 20 fields some of which have identical identifiers as those in the germplasm database, in order that the two databases can be combined to make rapid data searches or analysis. The data available on each accession varies from poor to very good, in most cases species identification and country of origin are available but precise collection locality coordinates and collection site altitude are available for only 75% and 55% of accessions. Ecogeographical data was provided for less than 20% of the accessions, however some of this data may provide future collectors with valuable information on soil types, associated vegetation and bedrock characteristics. The geographical distribution of each species has been mapped using appropriate mapping software and analysis of altitude ranges have been made, Clear differences in geographical distribution and altitude preferences could be established from this analysis but detailed ecological analysis was not carried out due to the shortage of data. Since the compilation of this database over 3000 additional unique accessions have been collected by ICARDA and information on these and future accessions will be added to the germplasm database. The development of a centralised database for wild relatives of crop species is clearly a desirable objective as its information can be made freely available to collectors, genebank curators, plant breeders and potential users. In a database form information on any particular field for a known species can be accessed rapidly and avoids the need to contact individual institutes or collectors. The data held on the wild wheat relative database can be supplied to individuals or institutes on request and it is hoped that regular updating of the database will occur.

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Microsatellites vs Isozymes markers: an application to the phylogenetic inference of brown trout (*Salmo trutta* L.) populations from Northern Italy

Presa P., Giuffra E. and Guyomard R., 1993.

Institute National de la Recherche Agronomique. Laboratoire de Génétique des Poissons. Domaine de Vilvert, 78352, Jouy en Josas. France.

Isozyme electrophoresis continues to be the most useful procedure to investigate the genetic variability of *Salmo trutta* populations; to date, stocks genetic characterization, enhancement programs streamlines and genetic management are based on this technique. One of the main advantages is its simple application, i.e. the systematic analysis of many loci and individuals with moderate costs.

However, due to the limitations imposed by certain technical constraints (number of variable loci and alleles per locus, set up of experimental conditions...), new genetic markers are needed in order to achieve a more precise estimation of the gene diversity of brown trout populations.

The advances in molecular biology techniques over the last decade have opened a direct way to genome analysis never thought before. Among the new genetic markers detected, short tandem repeated specific sequences (i.e. microsatellites) have been found in all the Vertebrate species so far analyzed, showing a high degree of polymorphism. This polymorphism has been detected at the intra and interpopulational levels and is being used in many species for genome mapping and linkage analysis studies.

No many attempts have been done up to now to compare the results obtained by the "old" and new techniques on the same populations and individuals, with the aim of validating the raising techniques.

Most of the knowledge about the genetic differentiation and restocking effects on natural populations of brown trout has been achieved using the enzymatic markers: assuming the consistency of these data, we have tried to validate the use of single microsatellite nuclear markers in the population genetics of this species.

Using the microsatellite markers we have inferred a preliminary phylogenetic relationships among 7 populations of brown trout from the Northern Italy complex, which have been already analyzed at the enzymatic level. The results are quite encouraging since the phylogenetic pattern obtained is close to that previously found at the enzymatic level using 47 protein-coding loci. It is worth to remark that only 4 microsatellites have been used to obtain this analogous result. In fact, the high number of alleles per locus and their unambiguous interpretation allow us to consider these microsatellite markers as a good tool to properly deal with Population Genetic studies at the intraspecific level.

Hordeum chilense as a source of resistance to fungal diseases.

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Wide hybridization provides a means to exploit the wide source of genetic variation and to study phylogenetic relationships. Crossing *Triticum* and *Secale* has produced the triticale, the first manmade cultivated allopolyploid. The interest in crossing *Triticum* and *Hordeum* is old. Hybrids have been produced, but their respective amphiploids were only obtained when wild species of barley were used. *H. chilense* is a wild barley that has interest in cereal breeding due to its high crossability with *Triticum*, *Hordeum*, and *Secale*. From crosses between *H. chilense* and *Triticum* spp. a wide range of "tritordeum" amphiploids have been produced that are proposed as a new crop. Also "hordecale" (*Hordeum* x *Secale*) has been suggested as a potential crop. These hybrids and amphiploids may be used as intermediates to transfer desirable traits to the cultivated cereals.

H. chilense is resistant to the leaf rusts of wheat, rye and barley (*P. recondita* ff. spp. *tritici* and *recondita*, *P. hordei*, respectively). Several mechanisms are responsible for that resistance. Mechanisms that hamper the fungi to find and to penetrate stomata have not been identified commonly in close relatives of wheat. *H. chilense* is till now the only case being documented of a close relative of wheat where wheat rust fungi have difficulty to find and recognise stomata.

All the *H. chilense* lines studied have been resistant to the isolates used of wheat, rye and barley powdery mildews (*Erysiphe graminis* ff.spp. *graminis*, *tritici* and *hordei*, respectively), and to *Septoria tritici*. *H. chilense* is also known to possess resistance to the aphid *Diuraphis noxia*, to the nematode *Meloidogyne naasi*, to the smuts, *Ustilago nuda* and *U. tritici*, and tolerance to salt. Some *H. chilense* lines are susceptible to *P. recondita* f.sp. *agropyri*, to the wheat and barley yellow rusts (*P. striiformis* ff.spp. *tritici* and *hordei*), to the wheat stem rust and to *Septoria nodorum*.

H. chilense could be a valuable source of resistance to wheat and barley diseases that might be incorporated in cultivated cereals. *H. chilense* resistance was found to be suppressed by the wheat genomes in the case of rust fungi. However, tritordeum is more resistant than wheat to *Septoria tritici*, *Erysiphe graminis*, *S. nodorum*, *Fusarium culmorum* and *Tilletia* spp.

EVALUATION OF AGRONOMIC CHARACTERS IN A COLLECTION OF
Lupinus albus L.

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To make a study of the variation of a series of interesting characteristics shown by the species may be of a great help for plant breeding programs.

The objective of this work has been to study the variation for a series of characteristics of interest to agronomists within a worldwide collection of 108 accessions of Lupinus albus L. by analyzing the amount of variation and the relationships among them, and based on this, to draw up an ideal plant model in order to achieve the best yield for every environmental conditions.

The collection has shown to be genetically variable for most of the studied characters; thus, this material is to be very useful for any L. albus breeding programm.

Relationships among characters have been analyzed with single correlations and PCA, complementing one each other in order to indicate the relative weight of every character on plant yield and, as a whole, the influence of every character on the other ones.

It would be interesting to get a type of plant with high yield on both main stem and first branches, searching for high number of pods and high grain weight genotypes.

Plant Genetic Resources Research in Czech Republic

Zdenek Stehno, Ladislav Dotlačil
Research Institute of Crop Production,
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Research of plant genetic resources (GR) in Czech Republic consists of four main activities: introduction, evaluation, documentation and maintenance.

Recently, the introduction of foreign genetic resources is carried out in a form of mutual exchange with other gene banks or in a form of purchase of new cultivars. Annually more than one thousand of GR is imported.

In the field of GR evaluation Gene bank cooperates with another 16 institutions in the republic. They are first of all research institutes, breeding stations and universities dealing with evaluation of crops or crop groups. Common information and results of evaluation they provide to documentation system carried on by Gene bank.

System of evaluation depends on the kind of crop, but main phases are common for most of crops. During preliminary evaluation first judgment and multiplication of material takes place. At the second step basic evaluation as a source of evaluation data is carried out. The samples receive in this phase national accession number as unique identification symbol. Only small parts of this sets proceed into the advanced phase of evaluation where emphasis on the characters most important for breeders, including yield is given.

National documentation system of plant genetic resources is named EVIGEZ. It consists of two parts gathering passport respectively evaluation data. Software of this system comes from Fox Pro 2.0 program. Data we receive from cooperating workplaces as record on paper form or on floppy discs.

Monitoring of the long-term storage facility in the Gene bank is another part of documentation system, which registers data on quantity, seed quality characteristics and location of seed samples in the store.

Long-term storage facility consists of five chambers having different temperature regimes. For active collection of seed propagated crops the temperature is kept on $+2^{\circ}$ C. Chambers for basic collection have regime -15° C.

During preparation phase purity and health state of seed samples are checked. After that, process of seed drying starts. Drying takes place under low temperature, not more than $+25^{\circ}$ C. When the moisture content reaches recommended level seeds are placed into glass jars with twist-off lids. The jars are placed into the long-term storage facility under code number.

Maintenance of vegetatively propagated plants is assured by cooperating workplaces, the same which are responsible for collection evaluation.

All activities mentioned above are coordinated by National Board on Plant Genetic Resources.

List of Invited Speakers

Lecture Course on
CONSERVATION AND USE OF GENETIC RESOURCES

List of Invited Speakers

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CONSERVATION AND USE OF GENETIC RESOURCES

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246 Workshop on Tolerance: Mechanisms and implications.

Organized by P. Marrack and C. Martínez-A. Lectures by H. von Boehmer, J. W. Kappler, C. Martínez-A., H. Waldmann, N. Le Douarin, J. Sprent, P. Matzinger, R. H. Schwartz, M. Weigert, A. Coutinho, C. C. Goodnow, A. L. DeFranco and P. Marrack.

247 Workshop on Pathogenesis-related Proteins in Plants.

Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoni, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:

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249 Workshop on Molecular Diagnosis of Cancer.

Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 Lecture Course on Approaches to Plant Development.

Organized by P. Puigdomènech and T. Nelson. Lectures by I. Sussex, R. S. Poethig, M. Delseny, M. Freeling, S. C. de Vries, J. H. Rothman, J. Modolell, F. Salamini, M. A. Estelle, J. M. Martínez Zapater, A. Spena, P. J. J. Hooykaas, T. Nelson, P. Puigdomènech and M. Pagès.

252 Curso Experimental de Electroforesis Bidimensional de Alta Resolución.

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Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.

Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening, J. Burgyn, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

254 Advanced Course on Biochemistry and Genetics of Yeast.

Organized by C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L. Calderón.

255 Workshop on The Reference Points in Evolution.

Organized by P. Alberch and G. A. Dover. Lectures by P. Alberch, P. Bateson, R. J. Britten, B. C. Clarke, S. Conway Morris, G. A. Dover, G. M. Edelman, R. Flavell, A. Fontdevila, A. García-Bellido, G. L. G. Miklos, C. Milstein, A. Moya, G. B. Müller, G. Oster, M. De Renzi, A. Seilacher, S. Stearns, E. S. Vrba, G. P. Wagner, D. B. Wake and A. Wilson.

256 Workshop on Chromatin Structure and Gene Expression.

Organized by F. Azorin, M. Beato and A. A. Travers. Lectures by F. Azorin, M. Beato, H. Cedar, R. Chalkley, M. E. A. Churchill, D. Clark, C. Crane-Robinson, J. A. Dabán, S. C. R. Elgin, M. Grunstein, G. L. Hager, W. Hörz, T. Koller, U. K. Laemmli, E. Di Mauro, D. Rhodes, T. J. Richmond, A. Ruiz-Carillo, R. T. Simpson, A. E. Sippel, J. M. Sogo, F. Thoma, A. A. Travers, J. Workman, O. Wrangé and C. Wu.

257 Lecture Course on Polyamines as modulators of Plant Development.

Organized by A. W. Galston and A. F. Tiburcio. Lectures by N. Bagni, J. A. Creus, E. B. Dumbroff, H. E. Flores, A. W. Galston, J. Martin-Tanguy, D. Serafini-Fracassini, R. D. Slocum, T. A. Smith and A. F. Tiburcio.

258 Workshop on Flower Development.

Organized by H. Saedler, J. P. Beltrán and J. Paz Ares. Lectures by P. Albersheim, J. P. Beltrán, E. Coen, G. W. Haughn, J. Leemans, E. Lifschitz, C. Martin, J. M. Martínez-Zapater, E. M. Meyerowitz, J. Paz-Ares, H. Saedler, C. P. Scutt, H. Sommer, R. D. Thompson and K. Tran Thahn Van.

259 Workshop on Transcription and Replication of Negative Strand RNA Viruses.

Organized by D. Kolakofsky and J. Ortín. Lectures by A. K. Banerjee, M. A. Billeter, P. Collins, M. T. Franze-Fernández, A. J. Hay, A. Ishihama, D. Kolakofsky, R. M. Krug, J. A. Melero, S. A. Moyer, J. Ortín, P. Palese, R. G. Paterson, A. Portela, M. Schubert, D. F. Summers, N. Tordo and G. W. Wertz.

260 Lecture Course Molecular Biology of the Rhizobium-Legume Symbiosis.

Organized by T. Ruiz-Argüeso. Lectures by T. Bisseling, P. Boistard, J. A. Downie, D. W. Emerich, J. Kijne, J. Olivares, T. Ruiz-Argüeso, F. Sánchez and H. P. Spaink.

261 Workshop The Regulation of Translation in Animal Virus-Infected Cells.

Organized by N. Sonenberg and L. Carrasco. Lectures by V. Agol, R. Bablanian, L. Carrasco, M. J. Clemens, E. Ehrenfeld, D. Etchison, R. F. Garry, J. W. B. Hershey, A. G. Hovanessian, R. J. Jackson, M. G. Katze, M. B. Mathews, W. C. Merrick, D. J. Rowlands, P. Sarnow, R. J. Schneider, A. J. Shatkin, N. Sonenberg, H. O. Voorma and E. Wimmer.

263 Lecture Course on the Polymerase Chain Reaction.

Organized by M. Perucho and E. Martínez-

Salas. Lectures by D. Gelfand, K. Hayashi, H. H. Kazazian, E. Martínez-Salas, M. McClelland, K. B. Mullis, C. Oste, M. Perucho and J. Sninsky.

264 Workshop on Yeast Transport and Energetics.

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265 Workshop on Adhesion Receptors in the Immune System.

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266 Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.

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267 Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.

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268 Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.

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Toro. Lectures by L. Adler, E. Blumwald, V. Conejero, W. Epstein, R. F. Gaber, P. M. Hasegawa, C. F. Higgins, C. J. Lamb, A. Läuchli, U. Lüttge, E. Padan, M. Pagès, U. Pick, J. A. Pintor-Toro, R. S. Quatrano, L. Reinhold, A. Rodríguez-Navarro, R. Serrano and R. G. Wyn Jones.

269 Workshop on Neural Control of Movement in Vertebrates.

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8 Workshop on the Diversity of the Immunoglobulin Superfamily.

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