ATSAF/IBPGR WORKSHOP ON
CONSERVATION OF PLANT GENETIC RESOURCES
Bonn, Germany, 6-9 May 1990

ATSAF
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Genetic diversity, and crop strategies for roots and tubers

ARBEITS-
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INTERNATIONAL
BOARD FOR
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Genetic diversity, and crop strategies for roots and tubers

Edited by Barbara Becker

ATSAF/IBPGR
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The Council for Tropical and Subtropical Agricultural Research (ATSAF e V) *is an association of scientists of German research institutions* The activities of the Council are concentrated on the following areas

- Advice to the Federal Ministry for Economic Cooperation the Federal Ministry of Food Agriculture and Forestry etc in their policies towards the CGIAR and research institutions supported by the CGIAR as well as other international agricultural research centers developing scientifically based criteria for support to developing countries
- Coordination of German Agricultural Research by mediating and fostering contacts between scientists from various research institutions
- Participation of German Agricultural Research in International Activities by maintaining close contact with the CGIAR system and its Technical Advisory Comm ttee (TAC) and by strengthening cooperation between German researchers and international agricultural research centers as well as national research institutions and research organizations in developing countries
- Public Relations by increasing public awareness on the importance of agricultural research for and in developing countries

The International Board for Plant Genetic Resources (IBPGR) is an autonomous international scient fic organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR) IBPGR was estabi shed by the CGIAR in 1974 The basic function of IBPGR is to promote and coordinate an international network of genetic resources centres to further the collecting conservation documentation evaluation and use of plant germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world Financial support for the core programme is provided by the Governments of Australia Austria Belgium Canada China Denmark France FRG India Italy Japan the Netherlands Norway Spain Sweden Switzerland the UK and the USA as well as the United Nations Development Programme and the World Bank

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CONTENTS

FOREWORD

In May 1990 a workshop was convened which brought together scientists from IBPGR and German researchers dealing with plant genetic resources The idea for this workshop had been born in September 1989 during an official visit of IBPGR s Director of Research Dr Alison McCusker to Mr Thomas Schurig then Head of the Department for Agriculture and Rural Development of the Federal Ministry for Economic Cooperation (BMZ) The objective of the workshop was to strengthen the relationship between IBPGR and German scientists by establishing research contacts and collaboration

The event was unprecedented for a couple of reasons For the first time five scientists from one single CGIAR centre had come to Germany for a research oriented workshop with German scientists Moreover since the workshop took place during the reunification process of the then still two Germanies it was the first time in ATSAF history that representatives of the former German Democratic Republic were able to take part in such a meeting whereas IBPGR has already had close links to researchers in the former German Democratic Republic for many years

After extensive preparatory discussions on the scientific focus of the meeting two distinct subjects were chosen

- **crop strategies for roots and tubers** (with special emphasis on cryopreservation) and
- **genetic diversity** (as a more general topic with specific research needs)

Both subjects play an important role in IBPGR s research programme both have special potential for collaboration between IBPGR and German scientists and institutions Furthermore both areas are highly relevant for developing conservation strategies for genetic resources of crop genepools

Each subject was given a full day of presentations discussion and working groups Professor Hans-Jorg Jacobsen kindly volunteered to chair the discussion about crop strategies for roots and tubers and Professor Gerhard Fischbeck about genetic diversity Professor Heinrich Weltzien then ATSAF Chairman commendably lead the opening session The workshop was complemented by an excursion of the IBPGR team to two German research institutions the Max-Planck-Institute for Resistance Breeding in Cologne and the Institute for Genetics of Bonn University

As intended the workshop has resulted in a number of specific proposals for collaborative research projects These proposals are remarkable because of the number of collaborators involved who took the opportunity provided by their workshop meeting to initiate these proposals Three research projects are presently under way as special projects

(i) **Refinement of Cryopreservation Techniques for Potato**
Cooperating partners FAL Braunschweig FRG (Dr G Mix-Wagner) DSM Braunschweig FRG (Dr H M Schumacher) University of Tubingen FRG (Dr L Schilde-Rentschler) IBPGR Rome Italy (*Dr L Withers*) CIP Lima Peru

(ii) **Effective Pollination Control Methods in the Regeneration of Germplasm**
Cooperating partners FAL Braunschweig FRG (Dr S Schittenhelm) ZiGuK Gatersleben FRG (Dr K Hammer) University of Halle FRG (*Dr M Dorn*) IBPGR Rome Italy (Dr R Rao)

(iii) **Spatial and Temporal Distribution of Genetic Diversity in Wild Forage Species under Stress Conditions**
Cooperating partners Botanical Garden and Botanical Museum Berlin-Dahlem FRG IBPGR Rome Italy ICRISAT Sahelian Centre Niamey Niger

This publication is a joint venture of IBPGR and ATSAF just as the whole workshop and the subsequent research projects The workshop was initiated and generously supported by BMZ The publication of the report would not have come true without the efforts of Ms Irmgard Berger and Ms Gabriele König of ATSAF secretariat who did the whole typing and layout of the report and helped with the editing The cover has been designed and printed by IBPGR Thanks are also due to Mr Brammer and the printing service of the Ministry of Food Agriculture and Forestry (BML) who printed the document at cost price

May this report further strengthen the relationship between IBPGR and the German research community Moreover may it contribute to the enhancement of plant genetic resources research and conservation which is our common objective and which was the ultimate reason for conducting the workshop

Dr Barbara Becker
ATSAF e V Liaison Officer
for Agricultural Research

Dr Hans-Joachim de Haas
Federal Ministry for Economic
Cooperation (BMZ)

Opening Adresses

A FEW REMARKS ON THE AGRICULTURAL RESEARCH AID FOCUS

H -Joachim DE HAAS

It is my privilege and pleasure to welcome you here on behalf of the Federal Minister for Economic Cooperation Dr Jurgen Warnke who has repeatedly stressed the necessity to combine ecological and economic aspects in our common struggle against hunger and poverty in Third World countries

May I - at the same time - use this opportunity to convey to you best wishes for a successful meeting from Mr Schurig[1] who most certainly has had his impact on getting this research-oriented meeting off the ground He sincerely regrets that he is unable to attend this gathering due to an additional engagement he has to fulfil these days

During these weeks of rapid changes in Europe and between the still two Germanies in particular it certainly needs mentioning that our engagement in international agricultural research is not going to decrease and will maintain its present and fairly substantial support in the foreseeable future - although the prospects of additional funds for our common endeavours are somewhat weak But continuing on the same level is already an achievement

I have been asked to contribute a few remarks on our targets and goals in supporting agricultural research activities

The Federal Government published its basic guidelines on development policies as early as April 1986 These guidelines refer to three major topics which need to be considered whenever support is to be granted

- ensuring sufficient food production on a national basis
- protecting and conserving natural resources
- combating poverty especially in rural areas

The second topic is of special interest to this meeting For a considerable time the Federal Republic of Germany has clearly demonstrated the importance given to this principle To a certain extent this can be judged from our support to IBPGR including our scientific cooperation with IBPGR and its Board of Trustees

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[1] then Head of Department for Agricultural and Rural Development Ministry for Economic Cooperation

Discussions like these had a strong influence on subsequent activities such as starting genebank projects in Ethiopia Costa Rica and Kenya These programmes most certainly were rather effective with regard to the basic establishment of such gene conservation centres However they are most likely less successful with regard to a liberal exchange of plant genetic materials from country to country and they are even less a success story with regard to their actual impact on national seed production programmes Nevertheless the long-term conservation of valuable indigenous seeds is in itself a commendable achievement

This in turn strengthens our interest in a well established and properly functioning IBPGR It is no secret that our support is going to continue as long as the present locality in Rome and close to FAO is maintained and the cooperation with FAO continues

We also know about IBPGR s keen interest in strengthening its own research activities Since we share this interest and envisage very useful results from such an additional programme our Ministry encouraged IBPGR and ATSAF to initiate this meeting The mere fact that so many scientists have come demonstrates the willingness of many institutes and scientists to cooperate with IBPGR in research programmes

Of course as a development agency the Ministry is not really in a position to support what many people call basic or fundamental research activities We have to stress that research programmes to be supported need to be close to applicability and to focus on actual problem solving

I am very confident that after two days of intensive discussions you will be able to present most valuable proposals for which we in turn will try to find the means of support Let me conclude by saying how glad I am to find so many scientists ready to establish closer links with IBPGR May I apologize for not being able to participate in the meeting since our department in the Ministry is affected by quite a few changes which will keep me extremely busy throughout the forthcoming weeks

IBPGR s RESEARCH PROGRAMME

Alison McCUSKER

Introducing IBPGR

Although IBPGR celebrated its 15th anniversary in 1989 a formal Research Programme was commenced only in 1987 A limited amount of research – mainly on seed physiology – has been funded since the late 1970s but the range of interests and the amount of activity has expanded very significantly in the past two or three years

The name International Board for Plant Genetic Resources has been retained but whereas IBPGR came into the world as a Board of experts serviced by a secretariat provided by FAO it has since become a Centre belonging to the Consultative Group on International Agricultural Research (CGIAR) responsible to the CGIAR through a Board of Trustees and financed by the CGIAR through a trust fund administered by FAO

There are 13 Centres in the CGIAR system four have their major research focus on commodity crops a further four concentrate on agricultural improvement in particular climatic zones two others are concerned respectively with livestock and animal diseases in Africa and three including IBPGR have worldwide mandates to study fairly restricted topics

In keeping with our worldwide mandate IBPGR is a worldwide Centre In addition to the Headquarters in Rome the Centre has seven Regional Offices (in Colombia Mexico Niger Kenya India and China the other being based at Headquarters) These offices form the main channels of communication between the Centre and national genetic resources programmes Their involvement in the research aspects of the programme has been minimal to date but there are plans to strengthen it considerably from this year onwards

The Research Focus

The overall objective of IBPGR s research effort is to provide information to strengthen the scientific and technological basis of collecting conservation and description of germplasm

On the recognized spectrum of research activities basic – strategic – adaptive – applied IBPGR focuses mainly on strategic and to some

extent on adaptive research Occasionally we uncover needs for basic research which generally cannot be pursued within IBPGR s core budget In most though not all cases applied research can be done by national programmes

Currently the main aspects of research for IBPGR are genetic diversity covering a whole range of activities from the molecular biology approach to the field survey and various aspects aimed at improving conservation science and technology Our staffing position on the research side reflects these interests There are six Research Officers three concerned with various aspects of the study and maintenance of diversity and three responsible for the research into conservation technology and safe movement of germplasm

In attempting to develop a sound basis of scientific knowledge for germplasm conservation IBPGR has an important role to fill within the CGIAR system where several of the other Centres are coming to recognize the increasing importance of utilizing diverse genepools in their crop improvement work Several CG Centres established Genetic Resources Units and proceeded to build up large collections of germplasm within their own programmes

IBPGR collaborates with some of these units to undertake research that makes use of their germplasm but is not wholly within their mandates or their priorities The CGIAR encourages Inter-Centre collaboration and IBPGR finds many opportunities in this regard

By contrast with our sister centres IBPGR does not maintain any germplasm collections at all Our function is to promote coordinate and assist germplasm conservation not to establish genebanks of our own In addition to addressing topics of widespread interest we focus much of our research effort on assisting national programmes to solve the scientific and technological problems associated with developing their genetic resources programmes

What are the key questions that need to be addressed by IBPGR? In broad terms we might sum up our task in just three questions

- What germplasm should be collected?
- How can it most effectively be conserved?
- How can it be made into an effective global resource for crop improvement?

Various members of the IBPGR staff will describe most of our activities during these two days of discussions Let me just now focus briefly on two which they will not cover

The first concerns seed storage After 15 years of concentrated activity by IBPGR and others to collect seed of traditional crop varieties threatened by the increasing use of improved varieties and wild species threatened by extensive clearing of natural vegetation to make way for farming and urban expansion the world now has a very considerable investment in stored seed It is part of IBPGR s responsibility to ensure that research is done to protect that investment

It is not surprising then that the improvement of seed storage technology is one of the main objectives of our research The method that IBPGR has recommended for the storage of seed – drying at 15 C and 15 % relative humidity followed by storage at –18 C in sealed packets – is effective but not economical Both the supply of electricity to maintain the recommended conditions for storage and the cost of it create serious difficulties for developing country national programmes IBPGR is therefore giving high priority to developing regimes that we believe will enable seeds to be stored safely at higher temperatures by first reducing them to lower moisture contents Any such regime must of course be very thoroughly tested before it could be recommended for general use Early results look very promising and the advantages of success will be enormous

The other area of research that I want to mention concerns proper documentation of the germplasm collected – which relies on proper identification of the material It may seem surprising that IBPGR would be involved in plant taxonomy and we have resisted it as far as possible but no germplasm acquisition will ever become an effective global resource unless it is first listed in a catalogue under an accurate name

Our practice has been to support taxonomic work only if the naming of germplasm accessions is a serious bottleneck to their conservation and/or use of genetic resources In that context for example we are currently funding a fairly substantial study in the genus *Allium*

However workers wishing to identify the secondary and tertiary genepools of crop species are often impeded not only by nomenclature but also by a lack of understanding of the phylogenetic relationships between crop species and their wild relatives This is an area of research which may seem on the face of it to be somewhat remote from the main focus of a CGIAR centre but there are still many unresolved taxonomic problems in the area of crop genetic resources and there is little interest by botanical institutions in addressing the agriculturally important taxonomic problems

IBPGR s approach to research is to identify recurring problems to seek the collaboration of experts in solving them and to transfer the results

to the sites of need The purpose of our visit to Bonn this week is to seek the collaboration of this group of German scientists together with others you may know in exploring areas of common interest in research that might help to solve some of the major problems in conserving and utilizing plant genetic resources to help feed the people of the world We suggested some particular projects for discussion but this gathering provides an ideal opportunity to explore much more widely the prospects for collaboration between us We look forward to two very fruitful days of discussion

Part I

Crop Strategies for Roots and Tubers

CROP STRATEGIES FOR ROOTS AND TUBERS POTATO – A MODEL FOR REFINEMENT YAM – A PROBLEM FOR DEVELOPMENT

Lyndsey A WITHERS

Introduction

The genetic resources of many important crop plants can be conserved by storage of their seeds at a low moisture content and at a low temperature (Seeds which survive under these conditions are termed orthodox) However there are a number of problem crops which cannot be conserved in this way They fall into two categories the recalcitrant seed-producing and the clonally propagated crops In the case of the former the seeds cannot tolerate drying and exposure to low temperatures in the case of the latter seed production is either absent or genetically irrelevant (e g due to high levels of heterozygosity) Both potato and yam are conserved as clones although not exclusively so

Until recently clones could only be conserved in the field genebank This method of genetic conservation can be unsafe because of risks of disease fire other accidents expense and management difficulties *In vitro* conservation the storage of clones in culture has been proposed as a safer alternative

In vitro Conservation

The central technologies of *in vitro* conservation relate to storage *per se* but this approach to conservation is a system that begins with germplasm collecting or acquisition followed by disease indexing/eradication and quarantine propagation storage and finally germplasm distribution for continued conservation or utilization (WITHERS 1989) By analogy with the seed genebank there is a need for both active and base storage *in vitro*

In vitro active storage can be carried out under conditions of slow growth in which cultures are maintained either at a reduced temperature or in the presence of growth inhibitors Appropriate methodology is well developed for everal crops including potato and cassava The latter has been chosen by IBPGR and CIAT as a suitable model upon which to test *in vitro* active genebank management standards and procedures (CHAVEZ *et al* 1987) Slow growth does however have limitations It is only applicable to shoot cultures and cannot on present

knowledge offer safe long-term (i e base) storage Thus for the other types of culture (ranging from protoplasts to cells callus and embryos) there is a need for both short to medium and long-term storage technology and for shoots an acceptable long-term option Cryopreservation (i e storage in liquid nitrogen) promises to meet these needs (WITHERS 1987)

Research has been carried out to develop cryopreservation methods for all types of culture and most success has been achieved for cell suspensions (WITHERS & KING 1980) A widely-applicable routine procedure involves pregrowth in a modified medium cryoprotection with a mixture of compounds slow cooling rapid thawing and recovery on semi-solid medium Difficulties are experienced in achieving successful cryopreservation for large organized structures such as shoots which would on the basis of their inherently low risk of genetic instability be the system of choice for conservation This problem hinders the wider application of cryopreservation One means of resolution may lie in an alternative culture system such as the somatic embryo IBPGR is currently pursuing the possibility of combining somatic embryogenesis artificial seed and cryopreservation technologies to find a new way of achieving the genetic conservation of recalcitrant seed-producing and clonally propagated crops

Strategies for Potato and Yam

Both potato and yam have been researched from the point of view of *in vitro* conservation although to different degrees of success attributable in part to differing levels of attention A large proportion (but by no means all) of this work has been carried out in the relevant CGIAR Centers CIP and IITA respectively In the case of potato virtually all of the components of an *in vitro* conservation scheme are in place including the widespread use of *in vitro* active storage by slow growth and *in vitro* exchange of germplasm (see MIX 1985 WITHERS 1989) However cryopreservation has not been developed to a level of reproducibility and wide applicability such that it could be recommended for use in a genebank

Over the last 10 – 15 years there have been a number of studies of the cryopreservation of potato shoot-tips and some notable advances made (see HENSHAW *et al* 1985 BENSON *et al* 1989 and references therein) These studies have highlighted the importance of achieving well-organized recovery and the necessity to carefully adjust culture conditions before and after cryopreservation Among the different studies there are significant variations in methodology and inconsistencies in response Nevertheless there is a substantial

foundation of work which if drawn together and combined with the advances made in recent years in the *in vitro* culture of potato and in cryopreservation in general would stand an excellent chance of success This would complete the portfolio of techniques for the *in vitro* conservation of what is both an important crop world-wide and an essential model for the application of *in vitro* conservation to other roots and tubers

Turning now to yam this can be propagated *in vitro* stored by slow growth and distributed in the form of cultures (NG & HAHN 1985) Techniques appear however to be less well developed and less widely practised than in the case of potato Cryopreservation of shoot cultures is untried that of a cell suspension of *Dioscorea deltoidea* is the only success on record for the genus (BUTENKO *et al* 1984) The development of *in vitro* conservation technologies for this crop would greatly benefit its safe conservation and wider more effective germplasm utilization

Yam is also of interest from another related point of view Conservation of germplasm does not involve a single problem and is not met by a single categorical answer To try to apply just one approach to the conservation of a crop genepool is over-simplistic and sure to prove inadequate There is a place for the appropriate application of *in situ* field genebank *in vitro* (both active and base) and seed conservation for a crop such as yam Pollen storage and in due course DNA storage should be considered also The complementary application of these different storage technologies should in the first instance be determined on the basis of knowledge of the genepool the possibilities of seed production the need for access to the genepool at different points in time and the consequent degree of emphasis to be placed on gene versus genotype conservation Superimposed upon this is the degree of security of the different storage modes and the level of development of the necessary technologies

It is further suggested here that by designing a complementary conservation strategy according to knowledge of the genepool and conservation and utilization needs and assessing the fitness of current technologies to carry out that strategy we can identify the research that remains to be carried out in order to achieve safe and efficient conservation Yam is considered to be a very suitable crop upon which to develop such a strategy in view of the balance of knowledge gained and still to be gathered and the susceptibility of its germplasm to a range of conservation technologies these factors being reinforced by the importance of yam as a subsistence crop in the developing world

For some other crops that are apparently well conserved including potato it is likely that revision of the balance of conservation technol-

ogies particularly between the storage of seeds and storage of clones would increase efficiency without hampering access to the genepool In all cases continual revision of the balance of technologies with time would be beneficial to ensure that technological advances be incorporated and current user needs met

References

BENSON E E HARDING K & SMITH H (1989) Variation in recovery of cryopreserved shoot-tips of *Solanum tuberosum* exposed to different pre- and post-freezing light regimes Cryoletters 10 323-344

BUTENKO R G POPOV A S VOLKOVA L A CHERNYAK N D & NOSOV A M (1984) Recovery of cell cultures and their biosynthetic capacity after storage of *Dioscorea deltoidea* and *Panax ginseng* in liquid nitrogen Plant Science Letters 33 285-292

CHAVEZ R ROCA W M & WILLIAMS J T (1987) IBPGR-CIAT collaborative project on a pilot *in vitro* active genebank FAO/IBPGR Plant Genetic Resources Newsletter 71 11-13

HENSHAW G G O HARA J F & STAMP J A (1985) Cryopreservation of potato meristems In Cryopreservation of plant cells and organs ed by KARTHA K K CRC Press Boca Raton 171-198

MIX G (1985) Preservation of old potato varieties In *In vitro* techniques propagation and long-term storage ed by SCHÄFER-MENUHR A Nijhoff/Junk for CEC Dordrecht 149-153

NG S Y & HAHN S K (1985) Application of tissue culture to tuber crops at IITA In Biotechnology in international agricultural research International Rice Research Institute Manila 29-40

WITHERS L A (1987) Long-term preservation of plant cells tissues and organs Oxford surveys of plant molecular and cell biology 4 221-272

WITHERS L A (1989) *In vitro* conservation and germplasm utilization In The use of plant genetic resources ed by BROWN A D H MARSHALL D R FRANKEL O H & WILLIAMS J T Cambridge University Press Cambridge 309-334

WITHERS L A & KING P J (1980) A simple freezing unit and cryopreservation method for plant cell suspensions Cryoletters 1 213-220

FUNDAMENTAL ASPECTS OF PLANT REGENERATION

Hans-Jorg JACOBSEN

Germplasm conservation *per se* does not require sophisticated cell and tissue culture technology since methods of *in vitro* storage are based on the use of easily regenerable plant tissues like meristems If however the genetic base in a given crop is limited due to extinction or loss of important material or if a certain trait (e g for a resistance or tolerance) is not available in the known accessions of a particular crop plant cell and tissue culture as well as molecular genetics may become a tool for selecting or creating new lines with the desired traits thus complementing the storage and evaluation of germplasm The general methods for the transformation of plants nowadays are well developed However apart from the characterization of important genes the regeneration of fertile plants from transformed or selected cell lines still remains a problem with a number of open questions

In most if not all crop species *in vitro* regeneration is far from being a routine if a particular crop species can be transformed and regenerated there are always cultivars which cannot be regenerated or only with low efficiencies (generally the interesting ones) Therefore we have to consider an important genotype problem In many crops however no efficient *in vitro* regeneration protocol is known due to the recalcitrance of the crop

In addition with many crop species we can obtain either high regeneration or high transformation rates when using *Agrobacterium tumefaciens* as a vector for gene delivery This implies considering alternative transformation technologies for most of the crop species Although these alternative methods such as direct gene transfer to protoplasts or particle bombardment (to name the most promising ones) are already in use a critical point has to be made

Most attention for establishing transformation and regeneration protocols has been payed to crops relevant to the agriculture in developed countries with the exception of rice So we lack particular expertise for crops relevant to developing countries This requires a higher input of research efforts at the international level on plant regeneration including research aimed at a better understanding of fundamental aspects of plant biology i e plant developmental biology at the molecular level

It is clear now that the establishment of regeneration protocols based on trial-and-error approaches often is a waste of time money and

scientific labour since in most cases success is rather unpredictable So one has to ask how well basic research is embedded into international programmes on crop improvement and whether or not the necessity to link applied with fundamental research has been recognized A possible solution for this problem could be the cooperation of the International Agricultural Research Centers or laboratories in developing countries – where the crops and their specific problems are well known – with laboratories in industrialized countries where traditionally more research on molecular aspects of plant developmental biology is carried out

IN VITRO PROPAGATION OF YAM AND PERSPECTIVES FOR ITS LONG-TERM CONSERVATION

Gunda MIX-WAGNER

Concerning *in vitro* preservation of plant genetic resources we are particularly interested in the root and tuber crops since storage of these species in the normal sense presents very great problems

At the Institute of Crop Science and Plant Breeding in Braunschweig we maintain an *in vitro* collection of 365 varieties and clones of potato and 40 genotypes of Jerusalem artichoke These varieties and genotypes can be stored for up to 2 - 3 years under minimal growth storage conditions

Recently we have begun to investigate *Dioscorea rotundata* (white yam) and *Colocasia esculenta* (cocoyam) The aim of our work is to develop a method for propagation and to test long-term storage conditions in order to create a way of maintaining collections of these species *in vitro* Up to now a number of research groups have been working with *Dioscorea* but only very few references have been published on work with *Colocasia*

In this paper I do not want to give a review of the available literature nor do I wish to give a description of the problems concerning these species Instead I only wish to give a preliminary report on the first results of our work

In general the main problem working with tropical species is to get the plant material to start with We have got some tubers of *Dioscorea rotundata* from Ghana So we started the investigation of *in vitro* propagation with tuber tissue

Tuber explant cultures

Figure 1 (p 18) gives a schematic description of the procedure for taking tuber explants into culture The tuber explants must have a piece of the outer skin Solution A (150 mg citric acid and 150 mg ascorbic acid/l) is used to avoid oxidation/blackening of the tissue Solution B (fungicide 5 3 g Benomyl/l and 3 handfuls of wood ashes) is added to eliminate fungi Adding solution C (1 % Ca-hypochlorite) is the common method to sterilize the tissue

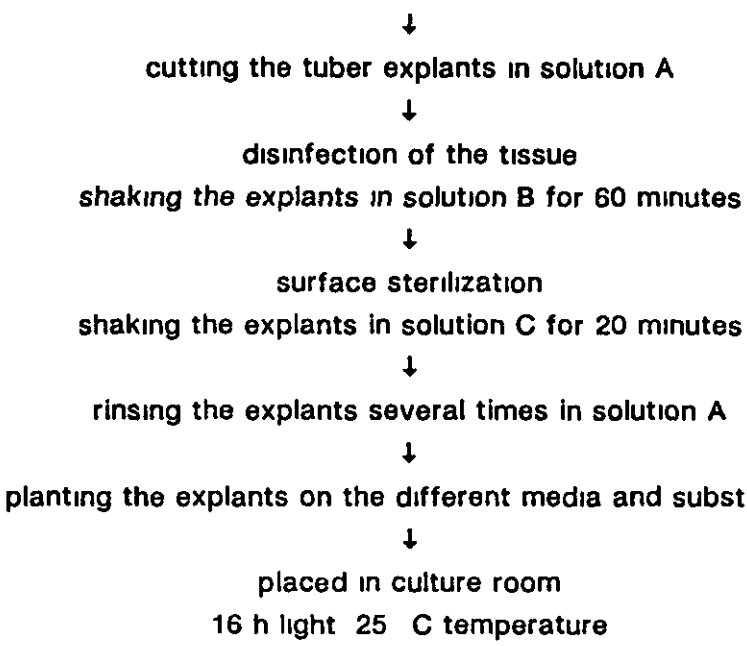


Figure 1 A schematic description of the procedure for taking tuber explants into culture

The two culture media used are based on MURASHIGE & SKOOG s basal medium but half concentrated Medium 1 is supplemented with 0 01 mg/l NAA 3 5 mg/l zeatine 30 mg/l cysteine 40 mg/l adenine sulfate and 20 g/l sucrose and medium 2 with 2 0 mg/l BA and 20 g/l sucrose The media were tested as solid media supplemented with agar and as liquid media with rock wool or Perlite to support the explants

The preliminary results are summarized in Table 1 The elimination of the fungi and bacteria was much more successful when solution B in combination with solution C was used rather than solution C alone It was much easier to obtain sterile tuber material of *Colocasia* than of *Dioscorea* after the treatment Without the use of solution A to avoid blackening all yam tuber explants turned brown The cocoyam tuber explants which were kept in solution A all the time showed nearly no blackening of the tissue

Looking at the regeneration figures it is not difficult to recognize that the explants cultured on rock wool or Perlite (liquid medium) showed a much higher rate of plantlet production than on agar

The *Colocasia* tuber explants showed a much better growth rate on medium 1 than on medium 2 For white yam it was just the other way round The explants cultured on medium 1 were able to regenerate more plantlets

Table 1 Preliminary results of the propagation of white yam and cocoyam

	Contamination		Blackening		Plantlet Regeneration	
	Solution B + C	Solution C	Control	Solution A	solid medium	liquid medium
white yam	60 %	95 %	100 %	10 %	med 1 30 % med 2 3 %	40 % 5 %
coco-yam	40 %	80 %	80 %	2 %	med 1 10 % med 2 30 %	55 % 90 %

Next Steps

The next steps of our investigations will be the development of plants from different tissues (e g explants from leaves stems veins) and research on the composition of the culture media for each tissue The aim is to get as many shoots as possible out of one explant

Perspectives for the Long-Term Storage Method

Here the aim is to make the sub-culture intervals as long as possible for the maintenance of the germplasm The following factors have to be tested to find out the best conditions for minimal growth storage

- environmental conditions
  * temperature
  * day length
  * light intensity
- culture conditions
  * solid/liquid media
  * addition of different compounds e g mannitol growth retardands
- plant material
  * plantlets
    micro tubers

PROTOPLAST FUSION AS A TECHNIQUE IN BREEDING OF POTATO AND OTHER TUBER CROPS

Lieselotte SCHILDE-RENTSCHLER & Helga NINNEMANN

Potato

Symmetric Protoplast Fusion

In 1974 when the first successful somatic hybrids were obtained by protoplast fusion (MELCHERS *et al* 1974) the utilization of this method for breeding purposes was emphasized

The modern cultivated potato is a highly heterozygous tetraploid crop Therefore breeding is difficult and time consuming Modern potato breeding takes advantage of the possibility to breed at the diploid level To reach the tetraploid level again WENZEL in 1979 already recommended the use of protoplast fusion Protoplast fusion allows the combination of two dihaploid clones selected for different traits without the rearrangement step of meiosis This is especially important for the combination of quantitatively inherited traits

Successful somatic hybridization was reported for wild species with cultivated potato (BARSBY *et al* 1984 HELGESON *et al* 1988 PEHU *et al* 1989) and a limited number of dihaploid breeding lines which were chosen because of their good response in cell culture (KARLSSON *et al* 1988 DEIMLING *et al* 1988 MASSON *et al* 1989) But only recently the method of protoplast fusion is entering potato breeding programmes The problem was to develop conditions which allow to isolate and culture protoplasts and regenerate plants for any breeding line routinely

The purpose of our project was the development of techniques for the application of somatic hybridization of regular dihaploid breeding lines including efficient fusion methods and identification of hybrids Breeding lines of four different companies and institutions were utilized A procedure was established which allowed to obtain plant regeneration from protoplasts for almost all breeding lines tested Optimizing electrofusion conditions resulted in fusion rates for double-fusion of 10-20 % routinely (SCHILDE-RENTSCHLER & NINNEMANN 1988) For the identification of hybrids isoenzyme analysis and RFLPs of the regenerating plants were used utilizing r-DNA-probes and non-radioactive labeling with dioxigenine (SCHWEIZER & HEMLEBEN 1988) In most cases digestion with one restriction enzyme gave sufficient information to discriminate the partners (Table 1)

Table 1 Determination of the most appropriate restriction enzyme for the identification of somatic hybrids of different breeding lines and wild species

For each clone combination the enzyme was chosen which gave typical bands for each of both partners The r-DNAs pRZ 52 and pRZ 83 (HEMLEBEN *et al* 1988) were utilized as probes A behind the pRZ-number means that one partner can be excluded only

combination of clones	restriction enzyme (probe pRZ 52 and pRZ 83)				
	EcoRV	DraI	BamHI	PSTI DraI	NsiI
R12		52			
1516			52		
1520		52			
1523		52		83	
1216		52			
0412		52			
0616		52	52		
0111				83	
0319		52 A		83 A	
R1V1		52		52	83
R1V2		52		52	83
0208				83	
0319			52		
NgrI	52				
Nneor		52			
Nspars		52			

More than 150 hybrids including 21 different breeding lines 3 wild species and 16 combinations were identified (Table 2 p 22) This material has been propagated *in vitro* and a sample sent to the breeders They have been increasing the clones in the greenhouse With the tubers obtained the evaluation of agronomic traits and resistances will be carried out in the field this year

As reported for other somatic hybrids we also found segregation of chloroplasts in somatic hybrids (Table 3 p 22) In most cases analysed so far a hybrid plant is provided with the chloroplasts of only one partner The situation for mitochondria has not yet been studied In a further project we will investigate the influence of the cytoplasmatic organells on the agronomic traits of the hybrids

Table 2 *Somatic hybrids* Number of different hybrids of 19 breeding lines and two wild species time of regeneration and % of abnormal plants for the different combinations are listed For the regenerates marked with * different feeding cultures were utilized

combination of lines	number of colonies analyzed	regeneration time (months)	number hybrids	% hybrids	% abnormal
R12*	390		21	5 4	50
R1V2	185	2–4	4	2 2	0
R1V1	167	2	0	0	
W1821*	ca 210	6	4	1 9	25
1520	209	4	28	13 4	50
0412	28	3	6	21 4	16 7
1523	127	2	3	2 4	
0111	113	2	28	24 8	0 4
0208	13	3	2	15 4	0
1516	116	3	30	25 9	3 2
0616	100	3	1	1 0	0
0106	113		0	0	
1216	137	2	16	11 7	12 5
N neor	13	3	9	69 2	11 1
N spars	11	6	5	45 5	0

Table 3 Distribution of chloroplasts in somatic hybrids In the case of R12* chloroplast-DNAs of both partners were present in the first test that of R2 in less concentration After several cycles of propagation only chloroplast-DNA of R1 could be detected

combination	number of hybrids with chloroplasts of partner a	partner b	partner a + b
R12	0	5	1
R1V2	3	0	1
W1821	3	2	0
0412	2	2	1
Nneor	2	5	1

Cybridization

Another area for which protoplast fusion can be used is the induction or removal of male sterility On the one hand male sterility is wanted for potato production from true seed on the other hand it is an obstacle for breeding It is caused by nucleus-cytoplasm interaction Therefore fusion of one partner protoplast of which the cytoplasm was inactivated with protoplasts of the partner in which the nuclei were inactivated leads to so-called cybrids with new nucleus-cytoplasm combinations (donor-recipient technique ZELCER *et al* 1978) In Table 4 (IWANAGA pers communication) selected dihaploid breeding lines with specific valuable traits from CIP are listed One of these shows good resistance to potato virus Y the other one to both potato leaf roll virus and virus Y These resistances were introduced from the wild species *Solanum stoloniferum* The interaction of *S stoloniferum* cytoplasm with *S tuberosum* nuclear genes leads to male sterility These clones therefore cannot be used as pollen donor By combination of the hybrid nucleus with different cytoplasms using the donor-recipient technique we should be able to overcome this crossing barrier

Table 4 Dihaploid breeding lines from CIP s breeding programme (IWANAGA personal communication)

cross number	resistance	cytoplasm	nucleus
V-3 2B	PVY	sto	tbr
V-3 30	PVY PLRV	sto	tbr

Asymmetric Protoplast Fusion

The utilization of germplasm resources for breeding purposes is very limited mainly because of the drawback in breeding level which is caused by the incorporation of the complete genome of the wild species or primitive cultivar In a future project we are trying to transfer parts of a genome only by asymmetric protoplast fusion

Other Tuber Crops

We are going to use the experience gained with potato in protoplast work for other crops one is sweet potato As can be seen from Figure 1 (p 24) crossing barriers exist between different relatives of

sweet potato Protoplast fusion could help to overcome these barriers and make the valuable traits in the related wild species available for breeding

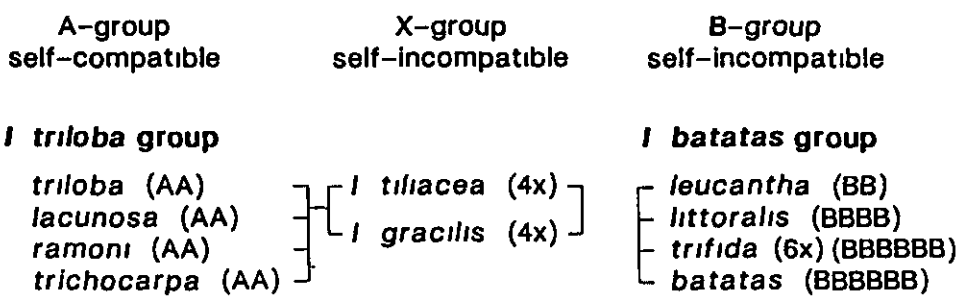


Figure 1 Sexual compatibility relationships in *Ipomoea batatas* section *batatas* (NISHIYAMA I 1982 p 267)

Legend —— compatible incompatible

The other crops we may be working with are the tuber bearing crops of the Andes besides potato Mainly three species exist which are important for the people in the highlands in South America *Ullucus tuberosus Oxalis tuberosa Tropaeolum tuberosum* Lately the germplasm which is still available is being stored in an *in vitro* gene-bank at San Marcos University in Lima Peru by R ESTRADA and his group We maintain a small sample of this collection in our laboratory No breeding has been carried out with these crops The Peruvian scientists are interested in using protoplast technology to increase variability since fertility is very low or nil In preliminary experiments with *Ullucus tuberosus* we found that protoplast isolation and cultivation seems to be no major problem Plant regeneration has not yet been carried out

In conclusion it can be said that in the case of potato protoplast fusion may help to utilize genetic resources more easily and to shorten the breeding process What has been elaborated for potato should also be possible for other less developed but – on a world scale – more important crops with similar problems if comparable effort is invested

References

BARSBY T L SHEPARD J F & KEMBLE R J (1984) Somatic hybridization in the genus *Solanum S tuberosum* and *S brevidens* Plant Cell Rep 3 165–167

DEIMLING S ZITZLSBERGER J & WENZEL G (1989) Somatic fusion for breeding of tetraploid potatoes Plant Breeding 101 181–189

HELGESON J P HABERLACH G T POHLMAN J & AUSTIN S (1988) Somatic fusion of *Solanum* species Plant Cell Tissue and Organ Culture 12 185–187

HEMLEBEN V GANAL M GERSTNER J SCHIEBEL K & TORRES R T (1988) Organization and length heterogeneity of plant ribosomal genes In Architecture of Eukaryotic Genes ed by KAHL G VHC Weinheim

KARLSSON S (1987) Somatic hybridization between anther derived dihaploid lines of *Solanum tuberosum* In Progress in Plant Protoplast Research ed by PUITE K J *et al* Kluwer Academic Publishers Dordrecht

MASSON J LANCELIN D BELLINI C LECERF M GUERCHE P & PELLETIER G (1989) Selection of somatic hybrids between diploid clones of potato (*Solanum tuberosum* L) transformed by direct gene transfer Theor Appl Genet 78 153–159

MELCHERS G & LABIB G (1974) Somatic hybridization of plants by fusion of protoplasts Mol Gen Genet 135 277–294

NISHIYAMA I (1982) Autohexaploid evolution of the sweet potato In Proceedings of the First International Symposium Sweet Potato ed by VILLAREAL R L & GRIGGS T D AVRDC Publication No 82-172 263–274

PEHU E KARP A MOORE K STEELE S DUNKLEY R & JONES M G K (1989) Molecular cytogenetic and morphological characterization of somatic hybrids of dihaploid *Solanum tuberosum* and diploid *S brevidens* Theor Appl Genet 78 696–704

SCHILDE-RENTSCHLER L & NINNEMANN H (1988) Kombination von Kartoffellinien durch Protoplastenfusion zur Regeneration tetraploider zuchterisch nutzbarer Hybridpflanzen Vortr Pflanzenzuchtung 14 149–163

SCHWEIZER G & HEMLEBEN V (1988) Charakterisierung spezifischer repetitiver Genomkomponenten in Kartoffelkultursorten Wildarten und deren somatischen Hybridpflanzen Vortr Pflanzenzuchtung 14 165–173

VILLAREAL R L (1982) Sweet Potato in the Tropics – Progress and Problems In Proceedings of the First International Symposium Sweet Potato ed by VILLAREAL R L & GRIGGS T D AVRDC Publication No 82-172 3–15

WENZEL G (1979) Neue Wege in der Kartoffelzuchtung Der Kartoffelbau 30 126–129

ZELCER A D AVIV D & GALUN E (1978) Interspecific transfer of cytoplasmic male sterility by fusion between protoplasts of normal *Nicotiana silvestris* and X-ray irradiated protoplasts of male sterile *N tabacum* Z Pflanzenphysiol 90 397–407

CRYOSTORAGE OF PLANT MATERIAL

Ursula SEITZ

In the course of work with *in vitro* cultures of plants the necessity has arisen for efficient storage methods This is particularly obvious in the case of cell suspension cultures that typically need to be transferred to fresh media every seven to ten days or in the case of shoot-tip cultures that are used for establishing germplasm banks in order to preserve the material over long periods of time

Cryopreservation i e storage at the temperature of liquid nitrogen is regarded as being the most suitable method for these purposes As far as we know the characteristics of plant cell cultures do not change during cryopreservation treatment This point will be discussed on the basis of biochemical capacities

The cryopreservation protocol used in our investigations corresponds with the method published by WITHERS & KING (1980) It comprises the following steps preculture in media supplemented with osmotically active compounds (mannitol sucrose etc) treatment with cryoprotective agents slow freezing storage at -196 C rapid thawing and post-thaw treatment and recovery growth Examples are presented here to show the possibilities of modifying this method and adapting it to various cell cultures More than 20 different species have been sucessfully cryopreserved during these studies

The *preculture treatment* is found to enhance freeze tolerance markedly The physiological events during this phase have not yet been investigated thoroughly but this treatment seems to induce a process which resembles stress hardening In the course of our studies we investigated the influence of several preculture additives on cell viability and freeze tolerance

The applicability of cryopreservation for storing meristems of important plant species and crop varieties will be discussed briefly on the basis of the literature published in this field

Conservation of Plant Cell Cultures with Special Characteristics

Daucus carota

Cells of an Afghan variety of *D carota* accumulate large amounts of anthocyanin The anthocyanin content of a culture which had been

frozen was compared with that of a control which had not The maximum values and the accumulation kinetics were both identical This is in complete agreement with the results published by DOUGHALL & WHITTEN (1980) who investigated the anthocyanin content in frozen-thawed carrot cells for the first time

Digitalis lanata

Cultivated cells of *D lanata* do not synthesize cardenolides *de novo* but they are able to transform added cardenolides (biotransformation) A typical reaction is the 12ß-hydroxylation of ß-methyldigitoxin Cryopreserved and control cultures showed the same time course in ß-methyldigitoxin production (SEITZ *et al* 1983) Moreover the final yields were also identical This was also valid after long-term storage (up to 4 years) Thus cell lines that have been selected for high productivity can be maintained in the frozen state

In this series of experiments two other plant species were investigated *Coleus blumei* (rosmarinic acid REUFF *et al* 1988) and *Panax ginseng* (ginsenosides SEITZ & REINHARD 1987) A summary of plant cell cultures that have been investigated after cryostorage with regard to biochemical capacities is given in Table 1 All of the frozen-thawed cultures mentioned there have been proved to retain their biochemical characteristics

Table 1 Freeze preservation of cell cultures with specific biochemical capacities

Species	Natural Compound	Reference
Catharanthus roseus	indole alkaloids	Chen *et al* (1984)
Chenopodium rubrum	betalaines	Ziebolz & Forche (1985)
Coleus blumei	rosmarinic acid	Reuff *et al* (1988)
Daucus carota	anthocyanin	Dougall & Whitten (1980) Seitz *et al* (1985)
Digitalis lanata	cardenolides (biotransformation)	Diettrich *et al* (1982) Seitz *et al* (1983)
Dioscorea deltoidea	steroids	Butenko *et al* (1984)
Eschscholtzia californica	benzophenanthridines	Ziebolz & Forche (1985)
Lavandula vera	biotin	Watanabe *et al* (1983)
Panax ginseng	ginsenosides	Seitz & Reinhard (1987)
Papaver bracteatum	chlorophylles	Ziebolz & Forche (1985)
Thalictrum rugosum	isoquinolin-alkaloids	Ziebolz & Forche (1985)

Cryopreservation Protocol

Many reports have shown that plant cells are only able to survive freeze–thaw processes if they have passed through a preculture treatment in a medium with enhanced osmolarity Consequently optimization of the protocol would always start at this point

Cell Viability during Preculture and Effectiveness of Various Additives

Digitalis lanata cultures were used for an extensive study of this subject *Digitalis* cells grown in normal culture medium showed only negligible changes in their viability rates over a 3-day period As a consequence of preculture treatments the viability curves were characterized by a marked decrease during the first 24 hours This was valid for trehalose mannitol sucrose and proline (Fig 1) and also for sorbitol (data not shown) The reduction in viability was transient in all cases where the cells were able to tolerate the treatment (trehalose mannitol sucrose melibiose) We consider this behaviour to be a consequence of stress which was then compensated for by adaptation to the enhanced molarity of the medium A more prolonged decrease in viability the production of phenolic compounds and a browning of the culture were observed in the presence of proline or sorbitol

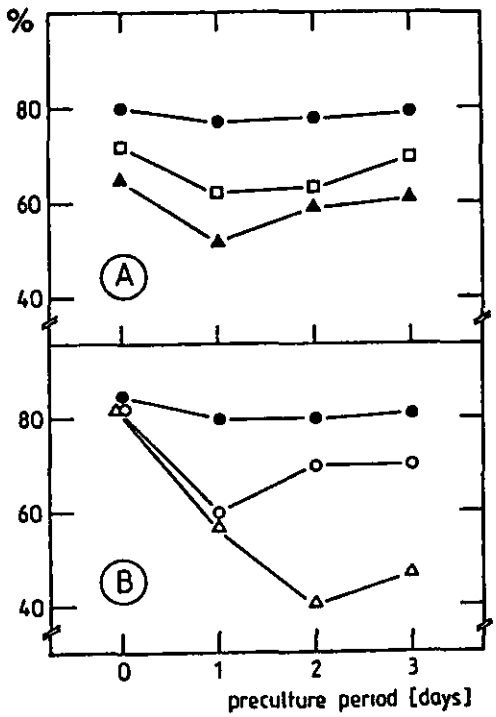


Figure 1

Viability of *Digitalis lanata* cells during a 3-day preculture period Concentration of preculture additives 0 3 M Fluorescein diacetate was used for viability tests

A control •
sucrose □
mannitol ▲

B control •
trehalose ○
proline △

In a further series of experiments we investigated the influence of several preculture additives on the freeze tolerance of *Digitalis* cells Besides the compounds which are commonly used as preculture additives such as mannitol we directed our attention to compounds which are accumulated in plants under stress conditions such as proline The best results were obtained when trehalose or mannitol were used (Fig 2) The post-thaw viabilities were 66 % and 53 % respectively Furthermore the cells were able to resume growth after a short lag period This is just one more example which demonstrates the broad applicability of mannitol Melibiose and sucrose were equally effective with *Digitalis* cultures Neither proline nor sorbitol proved suitable for this culture even though post-thaw viability was about 40 % In both cases the cells were not able to resume growth

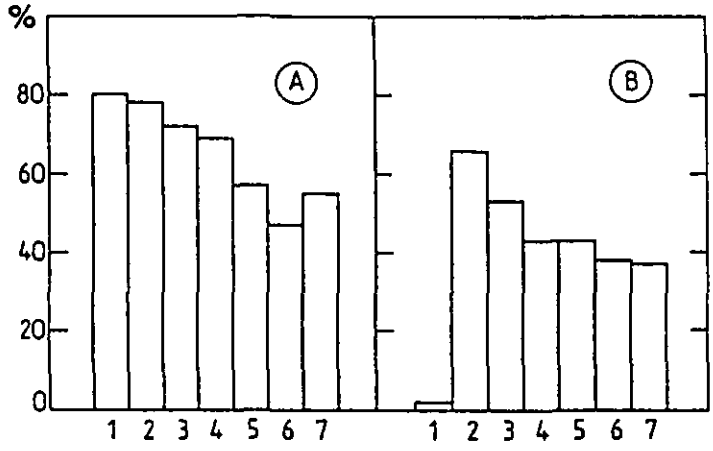


Figure 2 Viability (fluorescein diacetate) of *Digitalis lanata* cells at the end of the preculture period (A) and immediately after thawing (B) The following preculture additives (0 3 M) were used (2) trehalose (3) mannitol (4) sucrose (5) melibiose (6) proline (7) sorbitol (1) represents the control

Cryoprotectant Treatment

Without exception plant cells require chemical cryoprotection Mixtures are reported to be more suitable than single compounds DGS a mixture containing DMSO (0 5 M) glycerol (0 5 M) and sucrose (1 0 M) has been used successfully for a large number of species (WITHERS 1985) On the other hand we have found that with more than 10 different species including *D lanata* and *P ginseng* good results can be achieved with sucrose (1 0 M) as the sole cryoprotectant The replace-

ment of DMSO by 1 2-propandiol a treatment successful with recalcitrant seeds (BOUCAUD & CAMBECEDES 1988) was investigated using two *Atriplex* species (PGS Table 2) PGS and DGS proved to be of equal efficacy in terms of post-thaw viability The general applicability of this compound however should be confirmed using other plant species

Table 2 Comparison of two cryoprotectant mixtures in freezing experiments with *Atriplex litoralis* and *A hortensis* The percentage of viable cells was determined using fluorescein diacetate (A) at the end of preculture (B) after 1 h cryoprotection (C) immediately after thawing DGS DMSO glycerol sucrose PGS 1 2-propandiol glycerol sucrose

Species	A	B		C	
		DGS	PGS	DGS	PGS
A litoralis	92	80	78	54	52
A hortensis	94	80	82	52	47

The freezing regime storage conditions thawing and post-thaw treatments were as described previously (SEITZ & REINHARD 1987)

A list of plant species that have been cryopreserved in the form of cell cultures in our laboratory is shown in Table 3 Only those cultures are registered that are able to grow after they have passed through a freeze-thaw cycle

Table 3 Plant suspension cultures successfully cryopreserved (Tubingen 1989)

Atriplex hortensis	(Chenopodiaceae)
Atriplex litoralis	(Chenopodiaceae)
Atriplex patula	(Chenopodiaceae)
Atriplex semibaccata	(Chenopodiaceae)
Coleus blumei	(Lamiaceae)
Daucus carota	(Apiaceae)
Digitalis lanata	(Scrophulariaceae)
Digitalis purpurea	(Scrophulariaceae)
Panax ginseng	(Araliaceae)
Petroselinum crispum	(Apiaceae)

13 species from the following families

Acanthaceae
Aizoaceae
Apocynaceae
Celastraceae
Convolvulaceae
Oxalidaceae
Papaveraceae
Sterculiaceae

Cryopreservation of Meristems and Shoot-Tips

Until now I have concentrated my interest on plant cell cultures To give an impression of what has been done in the field of meristems I have compiled a list of published work (Table 4)

Table 4 Cryopreservation of meristems/shoot-tips

Species	Reference
Arachis hypogaea	BAJAJ (1979 1983)
Beta vulgaris	BROWN (1988)
Brassica napus	WITHERS *et al* (1988)
Cicer arietinum	KARTHA & GAMBORG (1978)
	BAJAJ (1979 1983)
Dianthus caryophyllus	SEIBERT (1976)
	SEIBERT & WETHERBEE (1977)
	UEMURA & SAKAI (1980)
	DEREUDDRE *et al* (1988)
Digitalis lanata	DIETTRICH *et al* (1987)
Fragaria x ananassa	KARTHA *et al* (1980)
	SAKAI *et al* (1978)
Malus domestica	KATANO *et al* (1983)
Manihot esculenta	KARTHA *et al* (1982)
	BAJAJ (1985)
Pisum sativum	KARTHA *et al* (1979)
Rubus spectabilis	REED (1988)
Solanum etuberosum	TOWILL (1981)
S goniocalyx	GROUT & HENSHAW (1978)
S tuberosum	BAJAJ (1985)
	GROUT & HENSHAW (1978)
	TOWILL (1983 1984)

In many cases the regeneration of plantlets or plants has been reported but it is not always clear whether the organised growth originated directly at the meristematic part or if a callus phase was included This is an extremely critical point for example in the case of potato since growth in the dedifferentiated state bears the risk of genetic instability Bearing this in mind I believe that it would be useful to investigate the cryopreservation of meristems using the following three approaches

1) **Cataloguing** Collection of all data available concerning cryopreservation of meristems (shoot-tips) Literature and unpublished work

2) **Screening** Intensive investigation of one or two important species (for example potato) with as many cultivars as possible with the aim of establishing a generally applicable protocol Investigation of the regeneration potential and organised growth Avoidance of the callus phase

3) **Plant Physiology** The aim is a better understanding of freeze adaptation in nature accumulation of sugars accumulation of compatible solutes role of abscisic acid etc Such knowledge could help us to find appropriate treatments

My co-workers during this project were Eva M GÖLDNER Doris BANSPACH and Ingrid REUFF The experiments were performed at *Pharmazeutisches Institut University of Tubingen* Auf der Morgenstelle 8 W-7400 Tubingen FRG

References

BAJAJ Y P S (1979) Freeze preservation of meristems of *Arachis hypogaea* and *Cicer arietinum* Indian J Exp Biol 17 1405-1407

BAJAJ Y P S (1983) Production of normal seeds from plants regenerated from the meristems of *Arachis hypogaea* and *Cicer arietinum* cryopreserved for 20 months *Euphytica* 32 425-430

BAJAJ Y P S (1985) Cryopreservation of germplasm of potato (*Solanum tuberosum* L) and cassava (*Manihot esculenta* CRANTZ) viability of excised meristems cryopreserved up to four years Indian J Exp Biol 23 285-287

BOUCAUD M -T & CAMBECEDES J (1988) The use of 1 2-propandiol for cryopreservation of recalcitrant seeds the model case of *Zea mays* imbibed seeds Cryo-Lett 9 94-101

BROWN A (1988) Cryopreservation of sugarbeet germplasm Plant Cell Tissue Organ Culture 14 161-168

BUTENKO R G POPOV A S VOLKOVA L A CHERNYAK N D & NOSOV A M (1984) Recovery of cell cultures and their biosynthetic capacity after storage of *Dioscorea deltoidea* and *Panax ginseng* cells in liquid nitrogen Plant Sci Lett 33 285–292

CHEN T H H KARTHA K K LEUNG N L KURZ W G W CHATSON K B & CONSTABEL F (1984) Cryopreservation of alkaloid-producing cell cultures of periwinkle (*Catharanthus roseus*) Plant Physiol 75 726–731

DEREUDDRE J FABRE J & BASSAGLIA C (1988) Resistance to freezing in liquid nitrogen of carnation (*Dianthus caryophyllus* L var Eolo) apical and axillary shoot tips excised from different aged *in vitro* plantlets Plant Cell Rep 7 170–173

DIETTRICH B POPOV A S PFEIFFER B NEUMANN D BUTENKO R & LUCKNER M (1982) Cryopreservation of *Digitalis lanata* cell cultures Planta Med 46 82–87

DIETTRICH B WOLF T BORMANN A POPOV A S BUTENKO R G & LUCKNER M (1987) Cryopreservation of *Digitalis lanata* shoot tips Planta Med 53 359–363

DOUGALL D K & WITTEN G H (1980) The ability of wild carrot cell cultures to retain their capacity for anthocyanin synthesis after storage at –140 (Planta Med Suppl 129–135

GROUT B W W & HINSHAW G G (1978) Freeze preservation of potato shoot-tip cultures Ann Bot 42 1227–1229

KARTHA K K & GAMBORG O L (1978) Meristem culture techniques in the production of disease-free plants and freeze-preservation of germplasm of tropical tuber crops and grain legumes In Diseases of Tropical Food Crops ed by MARAITE H H & MEYER J A Louvain-la-Neuve Belgium 267–283

KARTHA K K LEUNG N L & GAMBORG O L (1979) Freeze-preservation of pea meristems in liquid nitrogen and subsequent plant regeneration Plant Sci Lett 15 7–15

KARTHA K K LEUNG N L & PAHL K (1980) Cryopreservation of strawberry meristems and mass propagation of plantlets J Amer Soc Hortic Sci 105 481–484

KARTHA K K LEUNG N L & MROGINSKI L A (1982) *In vitro* growth responses and plant regeneration from cryopreserved meristems of cassava (*Manihot esculenta* CRANTZ) Z Pflanzenphysiol 107 133–140

KATANO M ISHIHARA A & SAKAI A (1983) A survival of dormant apple shoot tips after immersion in liquid nitrogen Hort Science 18 707–708

REED B M (1988) Cold acclimation as a method to improve survival of cryopreserved *Rubus* meristems Cryo-Lett 9 166–171

REUFF I SEITZ U ULBRICH B & REINHARD E (1988) Cryopreservation of *Coleus blumei* suspension and callus cultures J Plant Physiol 133 414–418

SAKAI A YAMAKAWA M SAKATA D HARADA T & YAKUWA T (1978) Development of a whole plant from an excised strawberry runner apex frozen to −196 C Low Temp Sci Ser B 36 31–38

SEIBERT M (1976) Shoot initiation from carnation shoot apices frozen to −196 C Science 191 1178–1179

SEIBERT M & WETHERBEE P J (1977) Increased survival and differentiation of frozen herbaceous plant organ cultures through cold treatment Plant Physiol 59 1043–1046

SEITZ U & REINHARD E (1987) Growth and ginsenoside patterns of cryopreserved *Panax ginseng* cell cultures J Plant Physiol 131 215–223

SEITZ U ALFERMANN A W & REINHARD E (1983) Stability of biotransformation capacity in *Digitalis lanata* cell cultures after cryogenic storage Plant Cell Rep 2 273–276

SEITZ U REUFF I & REINHARD E (1985) Cryopreservation of plant cell cultures In Primary and Secondary Metabolism of Plant Cell Cultures ed by NEUMANN K −H *et al* Springer Berlin/Heidelberg 323–333

TOWILL L E (1981) *Solanum etuberosum* A model for studying the cryobiology of shoot-tips in the tuber bearing *Solanum* species Plant Sci Lett 20 315–324

TOWILL L E (1983) Improved survival after cryogenic exposure of shoot tips derived from *in vitro* plantlet cultures of potato Cryobiology 20 567–573

TOWILL L E (1984) Survival at ultra-low temperatures of shoot tips from *Solanum tuberosum* groups Andigena Phureja Stenotomum Tuberosum and other tuber-bearing *Solanum* species Cryo-Lett 5 319–326

UEMURA M & SAKAI A (1980) Survival of carnation (*Dianthus caryophyllus* L) shoot apices frozen to the temperature of liquid nitrogen Plant Cell Physiol 21 85–94

WATANABE K MITSUDA H & YAMADA Y (1983) Retention of metabolic and differentiation potentials of green *Lavandula vera* callus after freeze-preservation Plant Cell Physiol 24 119–122

WITHERS L A (1985) Cryopreservation of cultured plant cells and protoplasts In Cryopreservation of Plant Cells and Organs ed by KARTHA K K CRC Press Boca Raton 243–264

WITHERS L A & KING P J (1980) A simple freezing unit and routine cryopreservation method for plant cell cultures Cryo-Lett 1 213–220

WITHERS L A BENSON E E & MARTIN M (1988) Cooling rate/culture medium interactions in the survival and structural stability of cryopreserved shoot-tips of *Brassica napus* Cryo-Lett 9 114–119

ZIEBOLZ B & FORCHE E (1985) Cryopreservation of plant cells to retain special attributes In Advances in Agriculture and Biotechnology ed by SCHAEFER-MENUHR A Kluwer Acad Publ Dordrecht

REPORT OF THE PLENARY DISCUSSION CROP STRATEGIES FOR ROOTS AND TUBERS

Results and recommendations

Lindsey A WITHERS

Clonally propagated crops including a number of roots and tubers of importance as staple foods in developing countries present serious problems in the achievement of their satisfactory genetic conservation Resolution of these problems would have significant human benefits The working group gave particular attention to potato as a relatively well-researched example in this category and to yam as a neglected one but other examples include sweet potato cassava cocoyam and taro

The genetic conservation of these crops relies heavily upon the use of the field genebank a conservation method which is expensive insecure in a number of respects and difficult to manage to adequately high standards The most serious deficiency for clonal crops is the lack of a true base storage method comparable to the low temperature storage of orthodox seeds The development of *in vitro* technologies is seen to offer many opportunities for improvement in the conservation of clones (including conservation under base storage conditions by cryopreservation in liquid nitrogen) for their safe movement in disease-free form and in due course their genetic improvement through biotechnological approaches

In terms of the development of *in vitro* conservation technologies potato is clearly the more mature crop of the two under consideration All components of an *in vitro* conservation strategy are in place with the exception of cryopreservation A number of research efforts carried out from the mid-1970 s yielded results that indicated the feasibility of cryopreservation for potato shoot-tip cultures but did not achieve a sufficient level of success in terms of breadth of applicability or consistency to encourage further development Nevertheless potato remains one of the crops for which cryopreservation has appeared the most promising The working group expressed the view that the diverse and discontinous threads of the past studies could fruitfully be pulled together to devise a programme of research that would capitalize upon the experience gained in those studies and draw upon the better knowledge that now exists in relevant areas of *in vitro* culture and cryobiology

Yam a clearly less mature crop from the point of view of its *in vitro* conservation and other aspects including knowledge of the genepool provided the working group with the opportunity to discuss some wider issues in the genetic conservation of clonal crops Conservation targets include both genes and genotypes For this reason seed conservation where possible has an important part to play as a means of conserving genes in their own right and as a back-up to clones Study of the genetic diversity of the crop genepool and the extent to which seed induction is possible would reveal the scope for taking this option and the need for further research to induce seed production For clones a balance should be struck between slow growth and (when available) cryopreservation Among the factors to consider here are feasibility safety and accessibility Again analysis would reveal research needs

A complementary conservation strategy that is based on a knowledge of genetic diversity and that is designed with the emphasis on needs rather than incidentally available technologies should be the objective for clonal crops Moreover where a distribution of sectors of the genepool of a crop e g potato or cassava has been based on criteria that are now out-of-date a revision along the lines suggested here would contribute to the safer *more efficient* genetic conservation of the crop Tasks of routine management and safety duplication might also be eased

Of the areas of research discussed by the working group the highest level of interest and the strongest basis for collaboration in terms of experience and facilities available emerged for the refinement of cryopreservation techniques for potato Drs G MIX L SCHILDE H M SCHUMACHER and U SEITZ expressed willingness to participate in such a collaborative effort Completion thus of a package of *in vitro* conservation technologies for potato would have an immediate application for the crop itself implementation can be foreseen in national programmes and the relevant International Center (CIP) Additionally it would add to the knowledge base on cryopreservation to the benefit of other clonal crops including the neglected yam

Although one specific initiative is highlighted here it is felt that it could provide the foundation for a more extensive longer-term involvement of *in vitro* conservation technologies for problem crops with associated opportunities for collaboration with developing country scientists and for training and technology transfer

Part II

Genetic Diversity

GENETIC DIVERSITY – INTRODUCTION

Alison McCUSKER

You will recall from my brief description of the IBPGR Research Programme that genetic diversity studies constitute a substantial part of it Half the staff are working in this area although we have not yet built up a comparable level of funding in our core budget That is one reason – the main reason – why we are giving some prominence to diversity proposals in seeking special project funding

Genetic diversity is a very crucial concept in genetic resources conservation and is very relevant to most aspects of the work of IBPGR It is in fact exactly what the IBPGR programme is all about

Let me take you back to the three basic questions

- What germplasm should be collected?
- What is the best way to conserve it?
- How can we make it an effective global resource?

There are problems in each of these three areas that can only be resolved by increasing our understanding of genetic diversity which is relevant to deciding

- how much material should be collected
- where and when it should be collected and
- how to plan the collecting programme so as to supplement rather than duplicate what has been collected before

A knowlege of the diversity of germplasm is also important for developing good management practices in genebanks – to determine for example

- how many of the accessions are in fact duplicates or nearly identical genetically
- whether gradual loss of viability of seeds is resulting in random loss of variation or selective loss i e whether there are selection pressures at work in the genebank eroding the variation we have so diligently collected
- how best to sample seeds from an accession for regeneration or distribution to users or
- how to ensure that *in vitro* conservation techniques are effective for long-term conservation

The measurement of genetic diversity is therefore a very important matter for IBPGR Many advances in basic research in this field have been made by the research community in recent years and there is a clear advantage for IBPGR to follow these developments closely and to ensure that new methods are improved and adapted for application in the genetic resources management context

Genetic diversity is a topic which creates an interface between two well-established fields of study namely genetics and ecology

I suppose you are aware that *in situ* conservation of genetic resources (or of biodiversity more generally) has recently received widespread attention by scientists and the general public IBPGR has not been involved directly in *in situ* conservation but in 1988 we signed a Memorandum of Understanding with the World Conservation Union IUCN agreeing on cooperation to further all matters of mutual interest to the two organizations in areas such as

- ensuring the compatibility of our programmes
- exchanging information and scientific data
- developing joint activities or
- working together on field activities as appropriate

It is in the area of genetic diversity studies that we believe there is much common ground between *ex situ* and *in situ* conservation and therefore between IBPGR and IUCN For example the same type of information about the distribution of genetic diversity in the field is necessary to plan collecting strategies and to select *in situ* conservation reserves Moreover the problems of conserving diversity and the effects which management practices might have on reducing it over time are probably just as great in the field as they are in the genebank and a thorough understanding of them is necessary to ensure long-term conservation by either method

Our present intention in IBPGR is not to become involved with *in situ* conservation directly but to aim for stronger collaboration with *in situ* conservation programmes to ensure a fully integrated approach to the conservation of crop genepools

Genetic diversity research as relevant to germplasm conservation involves some fairly new and important concepts and it is timely therefore for us to base a full day of our discussions on this topic

GENETIC VARIATION IN CROP SPECIES AND THEIR WILD RELATIVES A VIEWPOINT FOR THEIR CONSERVATION

Daniel G DEBOUCK

Introduction

The mandate of the International Board for Plant Genetic Resources (IBPGR) is to conserve the genetic diversity of useful crop species for present and future uses of plant breeders and agronomists (ANONYMOUS 1988) Being a centre of the Consultative Group on International Agricultural Research (CGIAR) its activities of research are related to germplasm collection characterization evaluation conservation and distribution as particular problems arise or as particular needs are expressed by the other CG centres for their commodity crops by national programmes worldwide and by the scientific community in plant genetic resources (PGR) in general I shall deal with some aspects of the raw material we are working with that is the genetic variation in crop species and their wild relatives dealing successively with some characteristics of genetic variation stressing on the interest of wild relatives of crops as p irt of this genetic variation suggesting eventually what could be an approach to conserve the genetic variation in order to fulfil IBPGR s mandate I will also enlist some of the areas of research where collaborative work might be particularly fruitful as it will appear evident that only strong collaborative work can cope with such a huge task

Characteristics of Genetic Variation

It would be vain to give a short definition of genetic variation in crop species as there might be as many definitions or interpretations of that phenomenon as living organisms we can observe around us There are however some attributes of genetic variation I shall illustrate with some examples as they orient our current activities for conservation Genetic diversity soon appears as unique indivisible dynamic and of unpredictable value

Uniqueness is perhaps best illustrated by genes of disease resistance in cereals where a stable resistance is often the result of a long built-up process of unique genes (CAUDERON *et al* 1973 DINOOR 1975 MOSEMAN *et al* 1984 PLUCKNETT *et al* 1987) and as observed by HARLAN (1978) a single gene can make the difference once you have found it As methods of easier gene transfer exist or will arise soon

(protoplast fusion genetic engineering) gene identification and availability will turn out to be the crucial questions This is the reason why germplasm collections *ex situ* or *in situ* which are well-studied and maintained and variable enough are so important

I shall illustrate the fact that genetic variation in crop species is indivisible and dynamic by the following examples It is often difficult to draw the line between the wild relatives and the crop species with accuracy most of our tree species can still be viewed as wild plants with a modified fruit The same statement is also valid for several herbaceous crops where the difference between the wild and the cultivated just depends on a couple of genes the translation of which into the phenotype has a very spectacular effect (GEPTS & DEBOUCK in press) Perhaps another illustration is found in the wild-weed-crop complex (HARLAN 1975) where a continuous gene flow between the wild and cultivated forms occurs which consequently leads to the genetic enrichment of both the wild and the cultivated materials (Table 1) This flow however is rare today (but of high significance for the future evolution of the crop and thus worth conserving and also in order to understand past events) as crops often are no longer in biological contact with their wild progenitors

Table 1 Reports of the presence of the wild-weed-crop complex

Crop species	Place	Reference
Chickpea	Karahbace Turkey	VAN DER MAESEN (1973)
Chili peppers	Central America	PICKERSGILL (1971)
Common bean	Junin Cuzco Peru	DEBOUCK *et al* (1989)
Lima bean	Cajamarca Peru	DEBOUCK *et al* (1987)
Maize	Huehuetenango Guatemala	WILKES (1977)
Potato	Potosi Bolivia	HAWKES (1977)
Rice	India and Thailand	OKA & CHANG (1961)

It seems very difficult to predict what will be the economic value of a very ordinary trait – and its genetic basis – over long periods of time Let us illustrate this observation with a few examples

1) H S GENTRY curious about plant evolution collected wild beans in Mexico in the 1960s (GENTRY 1969) SCHOONHOVEN and co-workers observed that only the wild beans were resistant to bean weevils but the cultivated beans were not (SCHOONHOVEN *et al* 1983) This resistance is due to the presence of a particular seed

protein variant (OSBORN 1988) unevenly distributed in the wild bean populations (OSBORN *et al* 1986) and not necessary for their survival (DEBOUCK 1989)

2) *Gossypium thurberi* TOD is a wild cotton distributed in Arizona Sonora and western Chihuahua (ANONYMOUS 1968) It has no lint however it gave lint strength to the upland cotton (HARLAN 1976)
3) *Lycopersicon hirsutum* a wild tomato from Ecuador and Peru has greenish fruits (RICK 1979) however it brings higher contents of ß-carotenes into the cultivated tomato and thus more colourful fruits (LATERROT 1989)

Again with tomatoes RICK (1976) mentions the case of transgressive variation where progenies of interspecific crosses outyielded expectations in comparison to the normal behaviour of the parents But this possibility has been little exploited so far

Interest in the Wild Relatives of Crops

As sources of agronomical characters In addition to the previous examples some reviews on this subject have been made (HARLAN 1976 HAWKES 1977) and one can see that there is practically none of the important crops where wild germplasm has not been used during the last sixty years (GOODMAN *et al* 1987) Some legume species have been left out but even there breeding approaches are changing (HARMSEN *et al* 1987) Breeders are thus progressively looking for interesting genes beyond the primary genepool that is the cultigen and its immediate wild relative(s) (HARLAN & DE WET 1971) This has been particularly true so far for the cultivated species of Poaceae (e g *Triticale* CAUDERON 1981) and Solanaceae (PATERSON *et al* 1988) but will probably be extended to other plant families As the lifespan of a plant variety is expected to be shorter and as integrated pest management procedures are to be extended due to increasing costs because of pollution one could reasonably expect an increasing use of alien germplasm in the next decades (PRESCOTT-ALLEN & PRESCOTT-ALLEN 1983 WILKES 1984)

As tools to understand evolutionary problems in crop species This is perhaps a less frequent use of the wild relatives of crop species by which we try to answer the following questions

- How did that particular crop arise?
- What is the amount of genetic diversity present in the crop *versus* in the whole plant species?
- Has the crop gained/lost additional genetic diversity during the course of the domestication process?

- How have the genepools evolved and which are the consequences for future breeding?

A recent example of this use has been given with *Phaseolus* beans where the origin of each cultigen has been established Until recently only four cultigens were usually referred to in literature (LEON 1987) but lately a fifth cultigen has been reported (DEBOUCK *et al* 1990) With the use of biochemical markers it has been possible to give a comprehensive explanation for apparently divergent data compiled from morphology and archaeology showing that genetic variation present in the cultivars is organized in genepools

- for the common bean	DEBOUCK & TOHME (1989) GEPTS *et al* (1986)
- for the lima bean	DEBOUCK *et al* (1989) MAQUET *et al* (1990)
- for the tepary bean	DEBOUCK (1989) SCHINKEL & GEPTS (1988)
- for *P polyanthus* bean	DEBOUCK *et al* (1990) SCHMIT & DEBOUCK (in press)

Similar findings have also been reported for rice (SECOND 1986)

These studies also allowed to improve our knowledge about the fraction of genetic diversity that has or has not been included during the domestication process A good appraisal of that balance – also known as founder effect (LADIZINSKY 1985) – is of definite importance when deciding about which part of genetic diversity should be collected/conserved particularly as far as the wild germplasm is concerned

Table 2 Quantitative estimation of the founder effect in *Phaseolus* beans by the number of variants in seed proteins

Species	Zone	Wild	Cult	Reference
P vulgaris	Mesoamerica	16	2	GEPTS *et al* (1986)
P vulgaris	Andes	13	7	DEBOUCK & TOHME (1989)
P lunatus	Mesoamerica	7	2	MAQUET *et al* (1990)
P lunatus	Andes	5	4	MAQUET *et al* (1990)
P acutifolius	Mesoamerica	25	2	SCHINKEL & GEPTS (1988)
P polyanthus	Mesoamerica	6	4	DEBOUCK *et al* (1990)

Similar observations about a reduction of variability during domestication have been done in other crops (chili peppers PICKERSGILL 1971

rice ENDO & MORISHIMA 1983 tomato RICK 1983) Out of the four conclusions reached by GOODMAN (1988) about the origin of maize three involved a certain kind of founder effect But it is certainly too early to conclude about a general applicability of this concept to most of our crops and much more detailed studies are needed (BROWN 1978) As there are still many evolutionary problems unsolved even for some of the most common crops (e g maize peanut) one could expect an increasing use of their wild relatives since they can be used as geographical markers (GEPTS 1990)

These studies – and many others particularly with the help of isozymes (wild barley NEVO *et al* 1979 wild bean KOENIG & GEPTS 1989 wild oat HAMRICK & ALLARD 1972 wild tomato RICK & FOBES 1975) also showed that the genetic variation is not uniformly distributed in a crop species spatially speaking that is some alleles are only regionally distributed It is tempting to think also about an uneven distribution of the alleles in time that is a variation from one year to another in certain populations according to their size as it is influenced by stresses (drought etc) but evidence is still scarce One can note that the evidences accumulated so far give rather a qualitative picture than a complete quantitative one of the allele distribution over the whole range of distribution of the wild relative of a crop species and its derived cultigen and on a reduced number of them too (HAMRICK *et al* 1979) A last point worth commenting is the likely influence of breeding systems and their variation on the genetic structure of natural populations (ALLARD 1975 BROWN 1978 HAMRICK *et al* 1979) an accurate knowledge of which seems to be a prerequisite for adequate regeneration and *in situ* conservation as well

Towards a Strategy for Conservation

Once we have considered these attributes of the genetic diversity of crop species we must conclude that we cannot separate the cultigen(s) from their wild relative(s) and that we have to consider them together in a broader conservation strategy

Some sort of germplasm exploration for using the PGR has been practised perhaps before the beginnings of agriculture during the plant gathering phase (REED 1977) But germplasm exploration for conservation (and this aspect is usually secondary to the utilization of germplasm) has been carried out on a large scale only during the course of this century This was due to an increasing awareness of the benefit of using more plant species and varieties (HARLAN 1984 SMITH 1986) and of the narrow genetic basis of plant varieties (ANONYMOUS 1972 HARLAN 1975) VAVILOV and his team launched a very broad pro-

gramme – some seventy expeditions and 400 research institutes – for the collection and study of PGR (HAWKES 1990) and this task was the starting point of hundreds of explorations around the world IBPGR recently helped in the collection of 170 000 additional samples for more than 130 species (PLUCKNETT *et al* 1987) principally focused on the land races and old varieties progressively wiped out by modern cultivars However it seems that the crop genetic diversity will perhaps never be completely collected and that the *ex situ* conservation will not be that kind of total food insurance contemplated at the beginning

Faced with increasing genetic erosion and pressures on natural resources (MYERS 1983) several ways of conserving diversity in plant organisms have now to be considered Although some kind of achievements can already be claimed for securing the land races of the most important crops (PLUCKNETT *et al* 1987) – but could it stand the comparison with VAVILOV s enterprise? – for the wild relatives there is still a long way ahead of us that could be oriented at

- defining the primary and secondary genepools of the different crops
- establishing the patterns of genetic diversity by defining
  - the potential distribution of the target species by herbarium surveys
  - * the actual distribution of the species by field explorations
  - * through genetic studies the frequence and distribution of alleles
  - * the dynamics of genetic diversity e g introgressive hybridization
- assessing risks of genetic erosion at population level

Once that information has been centralized for the different crop species it then becomes possible to design a synthetic approach of conservation linking the most traditional methods of *ex situ* and *in situ* conservation for efficiency reasons as they present complementary advantages (PRESCOTT-ALLEN & PRESCOTT-ALLEN 1983) the need for a co-evolution and for access being particularly relevant

Table 3 Characteristics/advantages of conservation methods

in situ	*ex situ*
Co-evolution	Access and use in breeding
Indirect costs	Direct costs
Recalcitrant seeds	Orthodox seeds
Perennial plants	Annual and short lived perennials
Low level of exchange	High level of exchange
Unsafe exchange	Safe exchange
Regeneration costs high	Regeneration costs low

It progressively becomes evident that *in situ* conservation should be considered through ecosystems conservation including as much living organisms as possible in order to allow the continuity of the evolutionary processes and also to have cost effective entities particularly over long periods of time (FRANKEL & SOULE 1981) The latter aspect concerns monitoring as from now onwards the conservation approach will not only be a synthetic one but also a dialectic one with periodical revisions of the germplasm status of the different target species and flexible responses When needed rescue collections could be organized where appropriate re-titutions could be planned

Conclusions

In order to be meaningful conservation methods should address a large fraction of the genetic variation present in crop species certainly beyond the primary genepool For crops other than the most common ones in the Poaceae and the Solanaceae the definition of genepools is still to be completed

In order to have some impact conservation methods should be flexible highranking first information about genetic erosion at the population level But together with the compilation of that information there is still a lot of basic information to gather about plant species distribution plant ecology and biology breeding systems etc with the help of ecogeographical surveys wherever necessary

In order to be useful to plant breeders conservation methods should primarily deal with all alleles known for a particular crop In this area there is still a large task ahead particularly in screening large populations of the wild relatives with the help of biochemical markers Some of these markers have proven to be useful but there are still important methodological problems to be solved for a better and true assessment of the genetic diversity present in a crop species Without the latter information it is not sure that any conservation effort will really fulfil its purpose

References

ALLARD R W (1975) The mating system and microevolution Genetics 79 115-126

ANONYMOUS (1968) Genetics and Cytology of Cotton 1956-67 139 1-84

ANONYMOUS (1972) Genetic vulnerability of major crops Washington D C National Academy of Sciences 307 pp

ANONYMOUS (1988) *Strategy of IBPGR International Board for Plant* Genetic Resources Rome Italy 17 pp

BROWN A H D (1978) Isozymes plant population genetic structure and genetic conservation Theor Appl Genet 52 145–157

CAUDERON Y (1981) Origine et évolution des triticales Indust Céréales 10 3–9

CAUDERON Y SAIGNE B & DAUGE M (1973) The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement 4th International Wheat Genetics Symposium Missouri Agric Exp Sta Columbia Mo USA 401–407

DEBOUCK D G (1989) Patrones de diversidad genética en *Phaseolus* hechos ideas e implicaciones CIAT Internal Seminars Centro International de Agricultura Tropical Cali Colombia

DEBOUCK D G GAMARRA FLORES M ORTIZ ARRIOLA V & TOHME J (1989) Presence of a wild-weed-crop complex in *Phaseolus vulgaris* L in Peru? Annu Rept Bean Improvement Coop 32 64–65

DEBOUCK D G LIÑAN JARA J H CAMPANA SIERRA A & DE LA CRUZ ROJAS J H (1987) Observations on the domestication of *Phaseolus lunatus* L Plant Genetic Resources Newsl 70 26–32

DEBOUCK D G MAQUET A & POSSO C E (1989) Biochemical evidence for two different gene pools in lima beans *Phaseolus lunatus* L Annu Rept Bean Improvement Coop 32 58–59

DEBOUCK D G SCHMIT V LIBREROS FERLA D & RAMIREZ H (1990) Biochemical evidence for a fifth cultigen within the genus *Phaseolus* Annu Rept Bean Improvement Coop 33 106–107

DEBOUCK D G & TOHME J (1989) Implications for bean breeders of studies on the origins of common beans *Phaseolus vulgaris* L In Current Topics in Breeding of Common Bean ed by BEEBE S Bean Program Centro Internacional de Agricultura Tropical Cali Colombia 3–42

DINOOR A (1975) Evaluation of sources of disease resistance In Crop Genetic Resources for Today and Tomorrow ed by FRANKEL O H & HAWKES J G Cambridge University Press Cambridge Great Britain 201–210

ENDO T & MORISHIMA H (1983) Rice In *Isozymes in Plant Genetics* and Breeding ed by TANKSLEY S D & ORTON T J Elsevier Amsterdam Holland 129–145

FRANKEL O H & SOULE M E (1981) Conservation and evolution Cambridge University Press Cambridge United Kingdom

GENTRY H S (1969) Origin of the common bean *Phaseolus vulgaris* Econ Bot 23(1) 55–69

GEPTS P (1990) Genetic diversity of seed storage proteins in plants In Plant Population Genetics Breeding and Genetic Resources ed by BROWN A H D CLEGG M T KAHLER A L & WEIR B S Sinauer Associates Inc Sunderland Massachusetts USA 64–82

GEPTS P & DEBOUCK D G (in press) Origin domestication and evolution of the common bean (*Phaseolus vulgaris* L) Common beans research for crop improvement Centro Internacional de Agricultura Tropical Cali Colombia

GEPTS P OSBORN T C RASHKA K & BLISS F A (1986) Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris* L) evidence for multiple centers of domestication Econ Bot 40(4) 451–468

GOODMAN M M (1988) The history and evolution of maize CRC Crit Rev Plant Sci 7(3) 197–220

GOODMAN R M HAUPLI H CROSSWAY A & KNAUF V C (1987) Gene transfer in crop improvement Science 236 48–54

HAMRICK J L & ALLARD R W (1972) Microgeographical variation in allozyme frequencies in *Avena barbata* Proc Nat Acad Sci 69(8) 2100–2104

HAMRICK J L LINHART Y B & MITTON J B (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants Ann Rev Ecol Syst 10 173–200

HARLAN J R (1975a) Crops and Man Foundations for Modern Crop Science Series American Society of Agronomy and Crop Science Society of America Madison Wisconsin USA

HARLAN J R (1975b) Our vanishing genetic resources Science 188 618–621

HARLAN J R (1976) Genetic resources in wild relatives of crops Crop Sci 16 329–333

HARLAN J R (1978) Sources of genetic defense Ann N Y Acad Sci 287 345–356

HARLAN J R (1984) Gene centers and gene utilization in American agriculture In Plant Genetic Resources – A Conservation Imperative ed by YEATMAN C W KAFTON D & WILKES G Westview Press Inc Boulder Colorado USA 111–129

HARLAN J R & DE WET J M J (1971) Toward a rational classification of cultivated plants Taxon 20(4) 509–517

HARMSEN R BLISS F A & OSBORN T C (1987) Breeding beans resistant to bruchids Annu Rept Bean Improvement Coop 30 44–45

HAWKES J G (1977) The importance of wild germplasm in plant breeding Euphytica 26 615–621

HAWKES J G (1990) N I Vavilov – The man and his work Biol J Linnean Soc 39(1) 3–6

KOENIG R & GEPTS P (1989) Allozyme diversity in wild *Phaseolus vulgaris* further evidence for two major centers of genetic diversity Theor Appl Genet 78 809–817

LADIZINSKY G (1985) Founder effect in crop-plant evolution Econ Bot 39(2) 191–199

LATERROT H (1989) La tomate Interet et utilisation des especes sauvages pour la creation varietale P H M – Revue horticole 295 3–7

LEON J (1987) Botánica de los cultivos tropicales Instituto Interamericano de Cooperación para la Agricultura Servicio Editorial San José Costa Rica

MAQUET A GUTIERREZ A & DEBOUCK D G (1990) Further biochemical evidence for the existence of two gene pools in lima beans Annu Rept Bean Improvement Coop 33 128–129

MOSEMAN J G NEVO E EL MORSHIDY M A & ZOHARY D (1984) Resistance of *Triticum dicoccoides* to infection with *Erysiphe graminis tritici* Euphytica 33 41–47

MYERS N (1983) A wealth of wild species – Storehouse for human welfare Westview Press Inc Boulder Colorado USA

NEVO E ZOHARY D BROWN A H D & HABER M (1979) Genetic diversity and environmental associations of wild barley *Hordeum spontaneum* in Israel Evolution 33(3) 815–833

OKA H I & CHANG W T (1961) Hybrid swarms between wild and cultivated rice species *Oryza perennis* and *O sativa* Evolution 15 418–430

OSBORN T C (1988) Genetic control of bean seed protein CRC Crit Rev Plant Sci 7(2) 93–116

OSBORN T C BLAKE T GEPTS P & BLISS F A (1986) Bean arcelin 2 Genetic variation inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris* L Theor Appl Genet 71(6) 847–855

PATERSON A H LANDER E S HEWITT J D PETERSON S LINCOLN S E & TANKSLEY S D (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction length polymorphisms Nature 335 721–726

PICKERSGILL B (1971) Relationships between weedy and cultivated forms in some species of chili peppers (genus *Capsicum*) Evolution 25 683–691

PLUCKNETT D L SMITH N J H WILLIAMS J T & ANISHETTY N M (1987) Gene Banks and the World s Food Princeton University Press Princeton New Jersey USA

PRESCOTT-ALLEN R & PRESCOTT-ALLEN C (1983) Genes from the wild – Using wild genetic resources for food and raw materials Earthscan International Institute for Environment and Development London UK

REED C A (1977) Origins of agriculture discussion and some conclusions In Origins of agriculture ed by REED C Mouton Publishers The Hague (The Netherlands) / Paris (France) 879–953

RICK C M (1976) Natural variability in wild species of *Lycopersicon* and its bearing on tomato breeding Genet Agr 30 249–259

RICK C M (1979) Potential improvement of tomatoes by controlled introgression of genes from wild species Proc Conf Broadening Genet Base *Crops* PUDOC Wageningen The Netherlands 167–173

RICK C M (1983) Tomato (*Lycopersicon*) Isozymes in plant genetics and breeding Elsevier Amsterdam Holland

RICK C M & FOBES J F (1975) Allozymes of Galapagos tomatoes polymorphism geographic distribution and affinities Evolution 29 443–457

SCHINKEL C & GEPTS P (1988) Phaseolin diversity in the tepary bean *Phaseolus acutifolius* A GRAY Plant Breeding 101 292–301

SCHMIT V & DEBOUCK D G (in press) Observations on the origin of *Phaseolus polyanthus* GREENMAN Econ Bot

SECOND G (1986) Isozymes and phylogenetic relationships in *Oryza* In Rice Genetics International Rice Research Institute Manila Philippines 27–39

SMITH N J H (1986) Botanic gardens and germplasm conservation Harold L Lyon Arboretum Lecture University of Hawaii Press Honolulu Hawaii USA 55 pp

VAN DER MAESEN L J G (1973) Genetic resources of chickpea Plant Genetic Resources Newsl 30 17–24

VAN SCHOONHOVEN A CARDONA C & VALOR J (1983) Resistance to the bean weevil and the Mexican bean weevil (Coleoptera Bruchidae) in non cultivated common bean accessions J Econ Entomol 76(6) 1255–1259

WILKES G (1984) Germplasm conservation toward the year 2000 potential for new crops and enhancement of present crops In Plant Genetic Resources – A Conservation Imperative ed by YEATMAN C W KAFTON D & WILKES G Westview Press Inc Boulder Colorado USA 131–164

WILKES H G (1977) Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize Econ Bot 31 254–293

POTENTIAL AND LIMITATIONS OF CURRENT METHODOLOGIES FOR INVESTIGATING GENETIC DIVERSITY

Toby HODGKIN

The collection characterization storage and improved utilization of plant genetic diversity are central to IBPGR s operations Currently three areas of research in genetic diversity of particular concern to IBPGR can be identified

- the way in which the biological ecological and environmental factors interact to produce the observed distribution of variation for target taxa
- the characters that can be used to describe genetically determined variation
- the procedures used to analyse the data and the statistics that most efficiently describe the observed genetic diversity

This paper is concerned with the kinds of characters that can be used to provide the raw data for genetic diversity studies and the advantages and disadvantages associated with their use

Four major types of characters which have been used in genetic diversity studies can be distinguished

- morphological characters and disease or pest resistances under the control of major genes – qualitative characters
- morphological characters (often of direct agronomic importance and relating to yield) controlled by polygenes – quantitative characters
- biochemical markers particularly seed proteins and isozymes which can be identified using some form of electrophoresis
- molecular genetic markers which identify variation at the DNA sequence level

Qualitative characters

The description of visible variation using easily detectable and simply inherited characters is or should be the starting point of any investigation of genetic diversity Such characters have been routinely included in IBPGR s Descriptor Lists and provide essential information on the material under study Most of the classic studies of variation by VAVILOV and his colleagues (e g SINSKAIA 1928 BARULINA 1930) relied heavily on such characters and these still provide valuable

descriptive information on patterns of variation in the species studied An example of the value of such data is the study by QUALSET (1975) of the correlation between resistance to barley yellow dwarf virus (BYDV) disease and major morphological characters in barley BYDV resistance was found to occur most frequently in Ethiopian land races with white or purple grain colour short rachilla hair and long glume awn Incidentally QUALSET was also aware of the importance of including environmental data in his studies and identified geographic location (Ethiopia) and elevation as major features affecting frequency of BYDV resistance This kind of information is of immense practical value in determining collection and utilization strategies

In the search for technically superior procedures the importance of this simple and directly useful information should not be neglected However it does not lend itself to any very formal analysis of genetic diversity In many species variation for many of the known marker genes may be absent over much of the material and the numbers of characters that can be studied is often extremely limited Genotype constitution is seldom known since most of the characters are controlled by dominant genes and heterozygote frequencies are unknown Moreover different genes may have the same morphological effect and hence be phenotypically indistinguishable Many of the known marker genes have a pronounced effect on vegetative growth and reproductive capacity which restricts their use in large-scale studies of diversity

Quantitatively inherited characters

For crop species and to a lesser extent their wild relatives the characters of most concern in breeding programmes are those which affect yield quality and performance These are usually determined by polygenes and have to be analysed using quantitative genetic procedures They are therefore quite unsuited to formal analyses of genetic diversity in so far as individual alleles cannot be identified or their effects measured Nevertheless because of their agricultural importance there have been a large number of studies of variation using such characters With the increased availability of multivariate analysis procedures a number of studies which seek to describe the variation of many characters in large numbers of accessions have been conducted For example SPAGNOLETTI-ZEULI & QUALSET (1987) measured eight characters in 3000 accessions of durum wheat and ERSKINE *et al* (1989) measured nine characters in 1 370 accessions of lentil In most cases geographic distribution of variation has been of particular concern although PEETERS (1988) has investigated the amount of diversity present in different countries for eight characters in barley *(Hordeum vulgare)* and a number of workers have explored

the correlation between environmental variables such as altitude and climate and yield characters

The information that such studies provide on diversity within populations is usually limited or absent (although JARADAT 1989 provides data on this) In fact most are not designed to give this information and the complex nature of the variates measured and the influence of the environment on their expression do not favour such an approach However such studies provide data on total variation in a crop or species for characters of direct interest to plant breeders and can provide information on the importance of major environmental variables such as altitude in determining character expression

Biochemical characters

Prior to the development of procedures for examining variation at the DNA level HUBBY & LEWONTIN (1966) noted that isozymes best fulfilled the criteria for population genetic studies The facts that their expression is normally unaffected by environment that they are simply inherited with codominant expression that individual alleles can easily be detected and methods for their assay are now quick and economical make them almost ideal markers of inter- and intrapopulation variation Compared with morphological gene markers larger numbers of loci can be surveyed although it should be remembered that some enzymes seem inherently more variable than others and that this may be reflected in the results obtained

Partly because of their nutritional importance seed storage proteins have also been the subject of a considerable number of investigations of genetic diversity (GEPTS 1990) In general higher levels of diversity are detected for seed storage proteins than isozymes This is not surprising the techniques used for analysis of seed protein diversity (SDS-PAGE) differ somewhat from those used for isozyme electrophoresis and detect different causes of polymorphism and seed storage proteins are encoded by small multigene families Generally however the advantages and disadvantages of investigating genetic diversity using seed storage proteins are similar to those for isozymes and the two can be considered together

Isozyme surveys have provided data on levels of genetic heterozygosity in target taxa and on outcrossing rates They have been used to demonstrate the adaptive nature of isozyme variation for example through the association of particular alleles or allele combinations with environmental factors and viability differences Thus NEVO *et al* (1988) described variation at 47 isozyme loci in *Triticum dicoccoides*

at Tabighe Israel and suggested that the distribution of alleles was structured according to aridity of the microsite tested On a larger scale WEEDEN *et al* (1988) surveyed 59 isozyme loci and morphological genes in 237 accessions of *Pisum sativum* from 17 countries He found high diversity levels considering the self-pollinated nature of the crop noted that coloured flower accessions contained most of the allozyme diversity and postulated that additional as yet uncollected variation probably existed in Turkey

Clonally propagated crops and species and perennials with a long generation time present particular problems for diversity analysis The standard procedures for estimation of genetically determined variation may not be available Progenies for analysis of inheritance may be difficult or impossible to obtain While the significance of isozyme band patterns should normally be confirmed by inheritance studies in yam phenotypic isozyme patterns have been used to provide preliminary data on the amount of variation present in different areas and cultivated groups and to assess the differences between different cultivated groups (HAMON 1988)

Despite obvious advantages some problems in the use of isozyme or protein variation should be noted First from a practical point of view the significance of allozyme diversity is not always clear Direct relationships between morphological characters and allozymes have only rarely if ever been determined It has been suggested that while isozymes are largely controlled by structural genes (DARMEVAL *et al* 1987) variation in agronomic characters is controlled by regulatory genes The relevance of isozyme variation to useful diversity is therefore not yet established On the other hand they do not fully reflect variation at the DNA level since only some base changes will result in amino acid changes of a type causing a change in mobility during electrophoresis

Molecular genetic markers

DNA sequence variation detected by molecular biological methods has been used to examine variation in single copy genes multigene families and organelle genomes (CLEGG 1990) Relatively few studies of variation in populations have yet been done using molecular techniques but their potential is frequently noted (e g BERNATZKY & TANKSLEY 1989) By comparison with isozyme studies much larger numbers of markers are available which can assay any component of the genome and detect any mutational change (e g in maize more than 800 markers have so far been mapped) The major disadvantages for genetic diversity analysis are that the assays are still relatively slow and

expensive and hence limited to rather small numbers Most procedures still rely on the use of radioactive chemicals which also restricts the widespread adoption of the techniques

RFLP maps based on polymorphism in single copy sequences have now been produced for maize tomato lettuce *Brassica* spp rice potato and soybean and are being developed for many other crops Their use in diversity studies however has so far been limited GEPTS & CLEGG (1989) surveyed 53 cultivated and 25 wild lines of pearl millet for polymorphism associated with the *Adh1* locus and found high levels of polymorphism in both wild and cultivated materials

To date the majority of surveys of plant molecular diversity have used the nuclear encoded ribosomal RNA gene family which is arranged in tandemly repeated blocks that occur at one or more chromosomal locations In practice the differences in DNA banding pattern for these genes which have been observed between plants or taxa require careful analysis because observed heterogeneity can have a number of causes (e g SAGHAI-MAROOF *et al* 1984) Generally however the results have shown that variation is present and that this approach has potential in more extensive diversity analyses

Much popular interest has been shown in the development of DNA fingerprints for use in the analysis of the human genome (JEFFREYS *et al* 1985) These fingerprints which result from variation in dispersed arrays of short tandem repeats have now been found in rice and other plant species (DALLAS 1988 ROGSTAD *et al* 1989) The genetic significance of pattern variation and similarity will require detailed analysis (LEWIN 1989 UITTERLINDEN *et al* 1989) but the technique may offer possibilities for analysis of clonally propagated material particularly where precise identification of duplicates in collections is required In fact IBPGR is already funding a visiting scientist to work in the pilot *in vitro* genebank project for cassava at CIAT and investigate the potential of DNA fingerprinting for characterisation and assessment of genetic stability in the material

IBPGR has already funded some research projects which involve the use of molecular genetic techniques The major focus of these projects has been the improved determination of species relationships by organelle genome analysis and data on intraspecific diversity in organelle DNA has been limited Other studies have detected intraspecific variation in cpDNA in some species (NEALE *et al* 1988 BANKS & BIRKY 1985) although GEPTS & CLEGG (1989) found none in pearl millet Analysis of variation at the DNA level has considerable theoretical attraction and will provide a versatile new set of techniques for investigating plant genetics However the expensive complex and cumbersome procedures suggest that its most appropriate use at least in the near

future will be to answer specific questions and not to conduct large surveys of variation

Table 1 Advantages and disadvantages of the above mentioned characters

Character	Advantages	Disadvantages
Simply inherited morphological markers	– obvious expression – simple inheritance – easy and quick to record	– small number – little variation – heterozygotes often undetected – can effect vegetative growth and reproductive ability
Quantitatively inherited characters of agronomic significance	– obvious expression – easy to record – large amount of variation – direct value in utilization	– genetic analysis complex – confounded by environmental effects – intrapopulation variation usually ignored – interaction with growth and reproduction
Biochemical markers	– individual alleles detected – simple inheritance – easily and quickly assayed – environmental effects not common	– only protein coding genes assayed – only variants causing mobility changes detected – limited number of loci available – relevance to useful variation uncertain
Molecular markers	– individual alleles detected – simple inheritance – no environmental effects – can detect changes throughout the genome	– expensive and complex – only small numbers can be assayed – relevance to useful variation unknown

Conclusions

IBPGR is concerned to ensure that the techniques used to investigate genetic variation will provide improved germplasm collecting characterisation conservation and utilization procedures It is unlikely that any one technique will be entirely satisfactory in this respect Improved measures of diversity based on molecular genetic or protein polymorphism may not adequately reflect morphological variation and at present molecular genetic techniques cannot be deployed on the scale required for the effective analysis of species diversity However a number of possibilities for the use of molecular genetic techniques exist such as the use of DNA fingerprinting in identifying duplicates the investigation of genetic similarity in clonally propagated crops and the study of species relationships

More generally research to extend the amount of information available from molecular genetic analyses is urgently needed as are studies which will provide fully comparable data on diversity using the different techniques available The most effective increase in the information needed to develop collection or conservation strategies will come from the combination of a variety of different techniques for describing diversity and no one technique should be regarded as replacing the others or making them obsolete

References

BANKS J A & BIRKY C W (1985) Chloroplast diversity is low in a wild plant *Lupinus texensis* Proc Natl Acad Sci USA 82 6950–6954

BARULINA H (1930) Lentils of the USSR and other countries Bull Appl Bot Genet and Plant Breed Suppl 40 265–304

BERNATSKY R & TANKSLEY S D (1989) Restriction fragments as molecular markers for germplasm evaluation and utilisation In The Use of Plant Genetic Resources ed by BROWN A H D FRANKEL O H MARSHALL D R & WILLIAMS J T Cambridge University Press Cambridge Great Britain 353–362

CLEGG M T (1990) Molecular diversity in plant populations In Plant Population Genetics Breeding and Genetic Resources ed by BROWN A H D CLEGG M T KAHLER A L & WEIR B S Sinauer Associates Inc Sunderland Mass USA 98–115

DALLAS J F (1988) Detection of DNA fingerprints of cultivated rice by hybridization with a human minisatellite DNA probe Proc Natl Acad Sci USA 85 6831–6835

DAMERVAL C HEBERT Y & DE VIENNE D (1987) Is the polymorphism of protein amounts related to phenotypic variability? A comparison of two-dimensional electrophoresis data with morphological traits in maize Theor Appl Genet 74 194–202

ERSKINE W ADHAM Y & HOLLY L (1989) Geographic distribution of variation in quantitative traits in a world lentil collection Euphytica 43 97–103

GEPTS P (1990) Genetic diversity of seed storage proteins in plants In Plant Population Genetics Breeding and Genetic Resources ed by BROWN A H D CLEGG M T KAHLER A L & WEIR B S Sinauer Associates Inc Sunderland Mass USA 64–82

GEPTS P & CLEGG M T (1989) Genetic diversity in pearl millet (*Pennisetum glaucum* (L) R BR) at the DNA sequence level J Hered 80 203–208

HAMON P (1988) Structure origine génetique des ignames cultivées du complexe *Dioscorea cayensis–rotundata* et domestication des ignames en Afrique de l Ouest Editions de l ORSTOM Institut Français de Recherche Scientifique pour le Développement en Coopération 223 pp

HUBBY J L & LEWONTIN R C (1966) A molecular approach to the study of genetic heterozygosity in natural populations In The Number of Alleles at Different Loci in *Drosophila pseudobscura* Genetics 59 577–594

JARADAT A A (1989) Ecotypes and genetic divergence among sympatrically distributed populations of *Hordeum vulgare* and *Hordeum spontaneum* from the xeric region of Jordan Theor Appl Genet 78 857–862

JEFFREYS A J WILSON V & THEIN S L (1985) Individual-specific fingerprints of human DNA Nature 315 76–79

LEWIN R (1989) Limits to DNA fingerprinting Science 243 1549–1551

NEALE D B SAGHAI-MAROOF M A ALLARD R W ZANG Q & JORGENSEN R A (1988) Chloroplast DNA diversity in populations of wild and cultivated barley Genetics 120 1105–1110

NEVO E BEILES A & KRUGMAN T (1988) Natural selection of allozyme polymorphisms a microgeographical differentiation by edaphic topographical and temporal factors in wild emmer wheat (*Triticum dicoccoides*) Theor Appl Genet 76 737–752

PEETERS J P (1988) The emergence of new centres of diversity evidence from barley Theor Appl Genet 76 17–24

QUALSET C O (1975) Sampling germplasm diversity in a center of diversity an example of disease resistance in Ethiopian barley In Crop Genetic Resources for Today and Tomorrow ed by FRANKEL O H & HAWKES J G Cambridge University Press Cambridge Great Britain 81–96

ROGSTAD S H PATTON J C & SCHAAL B A (1988) M13 repeat probe detects DNA minisatellite-like sequences in gymnosperms and angiosperms Proc Natl Acad Sci USA 85 9176–9178

SAGHAI-MAROOF M A SOLIMAN K M JORGENSEN R A & ALLARD R W (1984) Ribosomal DNA spacer-length polymorphisms in barley Mendelian inheritance chromosomal location and population dynamics Proc Natl Acad Sci USA 81 8014–8018

SINSKAIA E N (1928) The oleiferous plants and root crops of the family Cruciferae Bull Appl Genet and Plant Breed 19 555–619

SPAGNOLETTI-ZEULI P L & QUALSET C O (1987) Geographical diversity for quantitative spike characters in a world collection of durum wheat Crop Science 27 235–241

UITTERLINDEN A G SLAGBOOM P E KNOOK D L & VIJG J (1989) Two-dimensional DNA fingerprinting of human individuals Proc Natl Acad Sci USA 86 2742–2746

WEEDEN N F WOLKO B EMMO A C & BURR J (1988) Measurement of genetic diversity in pea accessions collected near the center of origin of domesticated pea IBPGR Unpublished Report 15 pp

PROBLEMS AND METHODOLOGIES FOR MANAGEMENT AND RETENTION OF GENETIC DIVERSITY IN GERMPLASM COLLECTIONS

V Ramanatha RAO

The following discussion is basically aimed at problems and methods of conservation of seeds with a view to preserve as far as possible the genetic structure of conserved accessions/populations In this type of static conservation the genotype or allele frequencies undergo some changes but loss of genes or alleles is minimized and contamination is avoided (FRANKEL & SOULE 1981)

The genetic diversity in a collection is eroded mainly due to the loss of seed quality in storage and loss of variation during regeneration

Loss of Seed Quality in Storage

The quality of seeds conserved is lost mainly due to the loss of viability the loss of vigour resulting in changes in development and yield and genetic effects on storability

These factors are largely influenced by storage conditions regeneration techniques applied and pre-harvest processing The differential survival of genotypes (ALLARD 1970) has been highlighted in the past however there is little precise evidence for this phenomenon It is generally believed that standard practices such as regenerating material when the viability drops by 5 to 10 % of the original germination level will counter most of these effects This underlines the importance of monitoring viability during storage

Loss of Variation during Regeneration

Regeneration of germplasm is an important area of genetic resources activity because conservation of the genetic structure of an accession as well as the retention of variation within an accession largely depend on this process The discussion that follows is largely drawn from the report of Prof E L BREESE (1989) whom IBPGR engaged as a consultant to review and evaluate the literature on regeneration One has to consider two major factors which influence the genetic structure of the conserved material during regeneration the frequency of regeneration and the regeneration methods

Both the frequency of regeneration and the regeneration methods may be crop-specific and will be difficult to generalize The frequency with which genebanks regenerate their material depends on the initial sample size the actual usage (demand to provide seed) and the length of seed viability

It is necessary to remember that however careful one is during regeneration further bottle-necking of material is inevitable A bottle-neck is an observable drop in the size of a population So the frequency of regeneration should be kept to a bare minimum Good conditions of long-term storage protect viability and therefore would considerably help in reducing the frequency of regeneration Generally one regeneration immediately after collecting or release from quarantine will not be enough However it is very essential that the initial seed increase should be as great as possible so that future regenerations can be reduced

Regeneration Methods

Integrity of an accession as reflected by the change in its genetic structure is affected by gene mutation outcrossing genetic drift genetic shift and contamination

The need in genetic resources is conserving adapted genotypes minimizing further evolutionary change (static conservation) until the desirable genes can be manipulated in developing better crop plants for the present and future In regeneration the important considerations are the following

In self-pollinating species the genetic and genotypic composition of the population is to be preserved Major limiting factors in preserving the genetic structure could be the population size and absence of selection during regeneration In the case of outcrossing species it is important to maintain the heterozygous/homozygous balance Here we need to have information on the life cycle reproductive biology and breeding system of the crop species under consideration However presently such information is seriously lacking for most crops and there is an urgent need to work on these aspects

Regeneration tactics are basically influenced by collection strategies A major problem is that the initial sample may include alleles with frequencies less than 0 05 Each regeneration would increase the bottle-necking process and the risk of losing such rare alleles is very high The level of outbreeding would greatly influence the tactics to be employed These could be

- avoiding contamination by alien pollen or seed through isolation and seed handling techniques
- minimizing drift or shift by manipulating the population size and by avoiding natural selection or
- pollination control (selfing/sibbing or securing random mating)

The genetic diversity of conserved material must be preserved during regeneration activity and this is more complex and difficult in the case of cross-pollinated crops than in inbreeders (PORCEDDU & JENKINS 1982)

Major Problems and Counteracting Measures

Mutations

The natural mutation rate though very small is an important consideration especially in ageing seeds and in the case of seeds with less than 80 % germination

One way to counter these effects is by regenerating material before there is serious loss in viability Direction of mutations mutation rate etc are thought to influence the differential survival of genotypes in heterogenous collections However there is no precise evidence and such effects have not been quantified More information through research on this aspect would help in planning better regeneration strategies

Pollen Adulteration

Even a moderate flow of foreign pollen can cause significant changes in the genetic structure of accessions Hence isolation techniques become a significant consideration during regeneration Isolation depends on methods of pollination and reproductive biology of the crop Isolation can be obtained by spatial/temporal barriers or natural/artificial barriers or hand crossing and bagging

When dealing with a large number of accessions regeneration in isolation is impractical though there is a rapid decrease in the rate of pollen contamination over distance Nevertheless in most crops less than 5 % cross pollination can occur over long distances depending on season location crop species direction of wind and species of vector and their preferences (RICHARDS 1986) Tall growing crops with islands of material being regenerated can act as an effective barrier Use of linen cloth or paper bags can further improve it However all

these should be considered on a species basis and would also depend on the effects of screening/bagging on the quality of seed

Most of the pollination control methods currently followed in regeneration of many crop species are based on intuition rather than experimental evidence Information on the extent of outcrossing effectiveness (including cost effectiveness) of different methods of isolation and their effect on seed yield and quality will considerably help in evolving suitable regeneration methods

Population Size and Genetic Drift

The sample size at collecting has a direct bearing on the number of plants to be grown for regeneration An accession would rarely come from a single plant In this case a small number of plants would satisfy the requirements for regeneration and the total number would mainly depend on the quantity of seed required However the number of plants sampled at collecting generally would be larger thus contributing to the heterogeneity of the accession and hence requiring the growing of a greater number of plants to retain the variation during regeneration

In small populations such as is the case with many of the genebank accessions to be regenerated random loss of alleles (genetic drift) can be very significant Effective population size (N) depends on the number of individuals contributing equally to the next generation Then the expected proportion of variation in a random mating population after t generations is equal to

$$(1 - \frac{1}{2N})^t$$

There will be about 1–2 % loss per generation with population size ranging from 25 to 50 Rare alleles are lost faster alleles with higher frequencies are preserved with greater confidence If one foresees more than 5 regenerations during the period of conservation of an accession N_e should then be much larger than 50 It is also important to use constant population size over generations

N_e is generally smaller than the actual population size (N) since not all the plants grown contribute to seed production due to chance or uncontrolled variation in the environment or genetic differences between plants The relationship between N and N due to these factors has been studied theoretically (CROW & KIMURA 1970) and in model situations (BRAY 1983 GALE & LAWRENCE 1984) However experimental evidence is lacking and additional research would be required to get more information on these aspects

If the differences between N and N_e are solely due to chance and/or environment then

$$N_e = (4N - 2) / (2 + V_{(k)})$$

where $V_{(k)}$ = variance in genetic contribution due to differences in fecundity and k = number of alleles

If the differences in fecundity are heritable then the formula becomes

$$N_e = 4N / [2 + (1 + 3h^2) V_{(k)}]$$

where h^2 = heritability

Based on the above calculations the following methods may be used while regenerating outcrossing species

1) Pair crossing with maximum control over generations

a) Pair crossing families kept distinct (variations chain crossing cyclical mating) This appears to be the most effective method since it eliminates differences due to chance environment and to some extent differences due to genetic constitution

$N_e = (4N - 2) / 2$ if N = 50 then N_e = 99

b) Mixing equal amounts of seed from each pair

$N = (4N - 2) / (2 + 2) = N - ½$ if N = 50 then N_e = 49 5

2) Controlled polycross hand crossing random females with mixed pollen

a) Maternal lines held as distinct subsets

$N = (4N - 2) / (2 + 1) = (4N - 2) / 3$ since $V_{(k)}$ is almost 1
if N = 50 then N ≅ 66

b) Equal amounts of seeds from maternal lines are bulked and maintained in a common container

$N \leq N$ if N = 50 then N_e is about 50 or less

3) Polycross with natural pollination equal quantities of seed pooled from each genotype/population

$N = (4N_mN_f) / (N_m + N_f)$ if N = 50 i e $N_m = N_f = 25$ then N = 50

assuming pollen comes from an equal number of plants (male)

4) Bulk-harvested naturally pollinated polycross

N may be close to N/2 if N = 50 then $N_e << 50$

Some of the problems with the above methods are

- Few attempts have been made to investigate the effects of variation in gametic contributions on effective population size This needs further investigation
- Variation in plant size and/or fecundity can potentially have significant effects on genetic drift by reducing N Usually this is countered by increasing the number of plants (N) well above the minimum required However quantifying the effects will considerably help to keep the numbers to the minimum necessary thereby reducing cost of regeneration
- The calculations involved in arriving at effective population size assume that the reproductive differences are due to random environmental variables In practical situations this is very unlikely especially so in the case of wild populations and heterogeneous land races Additional complications may arise due to level of ploidy
- The methods of regeneration described above are labour intensive and costly but there is no way around it This emphasizes the need for greater funding

Natural Selection and Genetic Shift

Natural selection depends on total fitness of individual genotypes It operates through differential survival and fecundity both of which may result in gene loss or change in gene frequencies So it is necessary to choose environments and cultural practices to reduce selection pressure during regeneration It is important to identify the major causes of differential survival and of G x E interactions which contribute to differential fecundity and to counter them Selection pressures may vary due to changes or differences between the original and new habitats However the suggestion that the accessions need to be regenerated close to the areas of their collection though ideal is usually impractical So due importance has to be given to ensure conditions that will

improve survival (temperature water requirements etc) improve reproduction (photoperiod sensitivity thermo sensitivity etc) and provide special conditions (protection against biotic and abiotic stresses pollinators)

Seed dormancy ageing and germination can affect an individual plant s contribution to seed production Therefore these factors also have to be considered during regeneration Similar consideration has to be given to competitiveness plant density and vernalization requirements Optimal cultural conditions are required to produce high quality seed a prerequisite for medium- and long-term conservation Much research needs to be carried out to find out about these conditions for most crop species When we consider their wild relatives almost nothing is known

Inbreeding Species

Most of the previous discussion centered around cross-pollinating crop species The majority of inbreeding species show genetic variation in floral morphology and mechanisms governing the degree of outbreeding which may be under the control of a small number of genes and highly influenced by the environment These do not show marked inbreeding depression and could be maintained as subsets of inbred lines or as bulk populations

Conclusions

In most genebanks very little consideration is given to the factors that have been discussed so far when planning for the regeneration of material In the past IBPGR has stressed the monitoring of viability to determine when the material should be regenerated However much less attention has been paid to how it should be done When we turned our attention to this matter recently we found that information about genetic diversity and breeding systems of accessions which would be necessary to provide guidelines on regeneration is sadly lacking

Much of the material collected 10–15 years ago when IBPGR began its activity is now becoming due for regeneration and therefore it is essential that research be undertaken and guidelines developed Otherwise we run the risk that genetic erosion will be just as serious in the regeneration plots as it is in the field/nature

References

ALLARD R W (1970) Population structure and sampling methods In *Genetic Resources in Plants – their Exploration and Conservation* ed by FRANKEL O H & BENNET E Blackwell Scientific Publications Oxford 97–107

BRAY R A (1983) Strategies for gene maintenance In Genetic Resources of Forage Plants ed by McIVOR J G & BRAY R A CSIRO Melbourne

BREESE E L (1989) Regeneration and Multiplication of Germplasm Resources in Seed Genebanks the Scientific Background IBPGR Rome

CROW J F & KIMURA M (1970) An Introduction to Population Genetics Theory Harper and Row New York

FRANKEL O H & SOULE M E (1981) Conservation and Evolution Cambridge University Press Cambridge

GALE J S & LAWRENCE M J (1984) The decay of variability In Crop Genetic Resources Conservation and Evaluation ed by HOLDEN J H W & WILLIAMS J T IBPGR George Allen & Unwin Ltd London/Boston

PORCEDDU E & JENKINS G (1982) Seed regeneration in cross-pollinated species Proceedings of the CEC/EUCARPIA Seminar Denmark 1981 A A Balkema Rotterdam 283–288

RICHARDS A J (1986) *Plant Breeding Systems* George Allen & Unwin Ltd London/Boston

CHARACTERIZATION OF GENETIC DIVERSITY WITHIN CORE COLLECTIONS BY RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs)

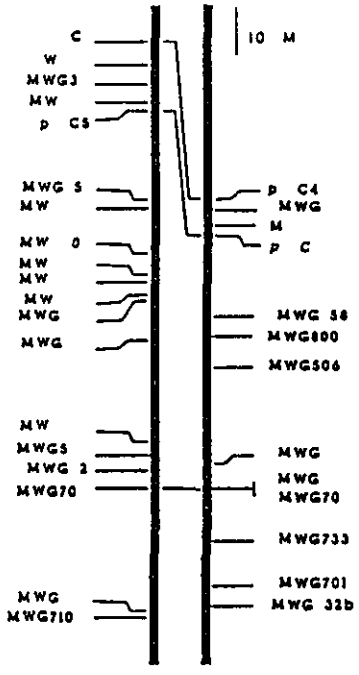
Gerhard FISCHBECK

Better use of genetic diversity stored in germplasm collections of cultivated plants can only be made if a proper level of evaluation of the accessions is reached Keeping in mind the huge number of accessions already stored in existing collections of some of the more important crop species such a requirement can only be met if a rational way of partitioning the work can be developed and followed To this end the concept of core collections was introduced by BROWN (1988) In essence a core collection should be extracted from the total number of available accessions in such a way that the limited number of core accessions reaches a representative level of genetic diversity compared to the total number of accessions

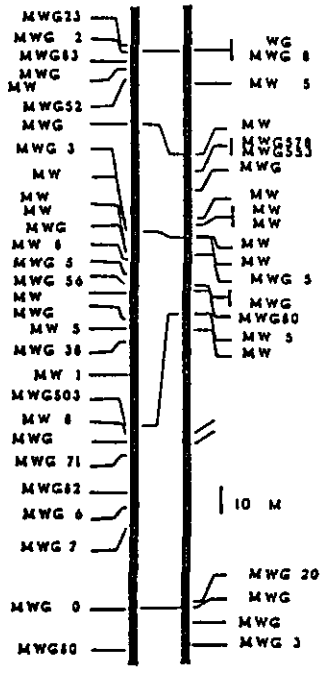
Based upon the results of several years of cooperation within the European Genetic Resources Programme which has been carried out under IBPGR guidance with special attention to barley a working group has been established and given the mandate to outline a programme for the establishment of a Barley Core Collection (BCC) The draft proposal of this programme envisages to extract a set of accessions which does not exceed 2 000 of the estimated total of about 100 000 barley accessions maintained in genetic resources collections around the world It is intended to discuss the proposal with the international community of barley workers during the barley genetics congress to be held in 1991 in Helsingør with the intention to finalize the programme and put it into action

During the last decade a significant part of molecular genetics research with cultivated plants has been devoted to develop genetic markers based upon the polymorphism obtained from cloned DNA probes upon hybridization with DNA extracted from different genotypes and the treatment with a set of restriction enzymes (JAHOOR *et al* 1990 BERNATZKY & TANKSLEY 1986 HELENTJARIS 1987)

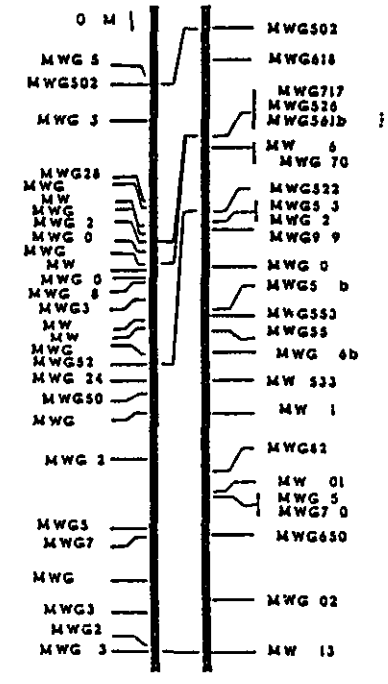
With the support of the Federal Ministry for Research and Technology (BMFT) a cooperative effort is being made by the Institute for Botany (Prof HERRMANN) Munich the Institute for Resistance Genetics (Prof WENZEL) Grunbach and the Department for Plant Production and Plant Breeding (Prof FISCHBECK) Freising-Weihenstephan to establish a set of RFLP markers for the barley genome The result of these efforts is shown in Figure 1 (p 70/71 – replacing the preliminary table



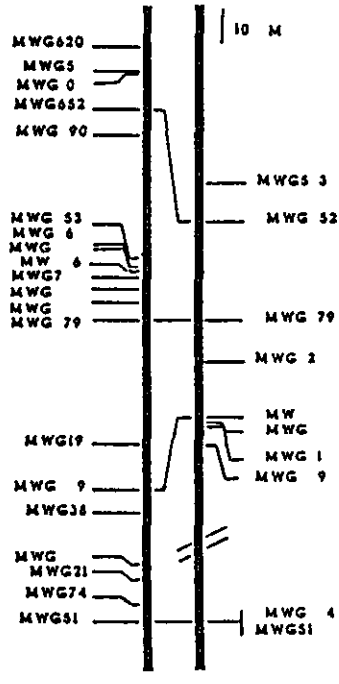
1H (152 cM)



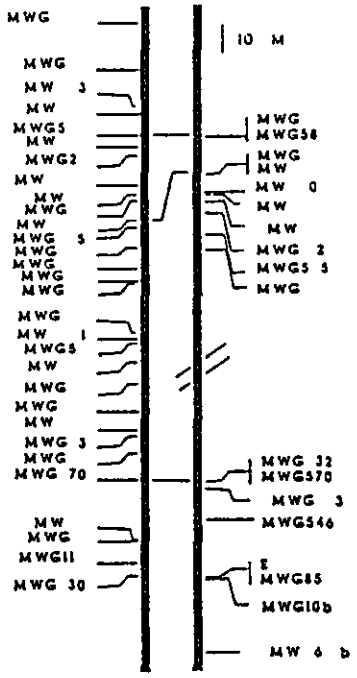
2H (230 cM)



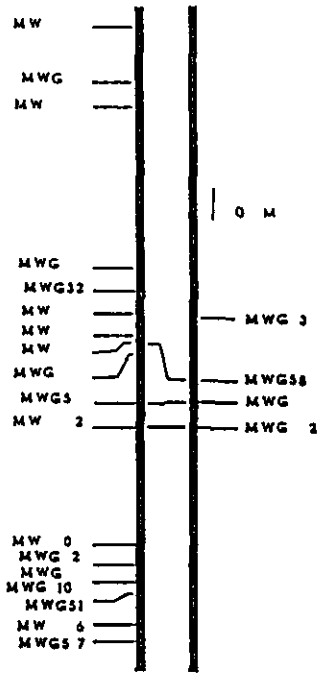
5H (245 cM)



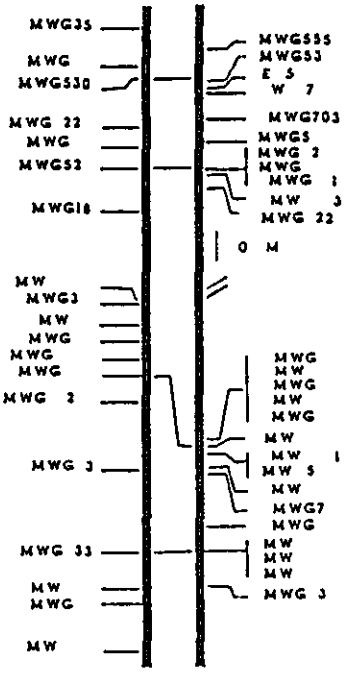
6H (174 cM)



3H (254 cM)



4H (180 cM)



7H (218 cM)

Figure 1

RFLP map of the barley genome For each chromosome the DH-derived map is shown on the right the F_2/F_3-derived map on the left bar Chromosomes are oriented with the short arm on top The positions of common references are marked by lines between the bars and gaps are indicated by inclined lines Vertically oriented dotted lines mark regions of distorted segregation

(GRANER JAHOOR *et al* 1991 p 253)

presented at the workshop) The marker set mainly consists of single copy and low copy DNA clones The 251 clones listed in Figure 1 have been assigned to specific chromosomes of the barley genome by hybridisation with a set of wheat-barley addition lines The mapping of genetic linkages between the markers located on the same chromosome is based upon segregation data obtained from the F_2 population of two barley crosses

Most likely the polymorphisms which can be detected with such a set of probes will not be randomly distributed but will indicate genetic similarities as well as genetic diversity if applied e g to a set of barley cultivars Therefore it may be helpful to select for a maximum degree of genetic diversity between prospective crossing parents even before linkage relationships with economically important characters are established which eventually will also include quantitative trait linked loci (QTL)

Within the near future it should be possible to select a set of 28 probes which are not closely linked but located on the same arm of each of the seven barley chromosomes and which are known to reveal restriction fragment polymorphism between commercial barley cultivars It certainly would be extremely informative to use such a set of markers not only to determine but also to quantify the genetic diversity which will be *present in the future* barley core collection since such data may provide sufficient information to develop a characterization system for genetic diversity in barley

References

BROWN A H D (1988) The case for core collection In The Use of Plant Genetic Resources ed by BROWN A H D FRANKEL O H MARSHALL D R & WILLIAMS J T Cambridge University Press Cambridge Great Britain 136

BERNATZKY R & TANKSLEY S D (1986) Majority of random cDNA clones correspond to single loci in the tomato genome Mol Gen Genet 203 8–14

GRANER A JAHOOR A SCHONDELMAIER J SIEDLER H PILLEN K FISCHBECK G WENZEL G HERRMANN R G (1991) Construction of an RFLP map of barley In Theor Appl Genet 83 250–256

HELENTJARIS T (1987) A genetic linkage map for maize based on RFLPs Trends in Genet 3 217–221

JAHOOR A *et al* (1990) Development of RFLP markers for the barley genome In Vortrage fur Pflanzenzuchtung der Arge Pflanzenzuchtung 18 104–121

CHANGING PRIORITIES IN CONSERVATION OBJECTIVES OF GENETIC RESOURCES

Hans-Rolf GREGORIUS

General Deliberations

This short communication is not intended to be a representative report and evaluation of changes in the objectives of conservation of genetic resources but will rather try to make a short and concise statement on why such changes are required and in which direction they could proceed For this purpose it is helpful to start with brief definitions of some basic terms in the sense in which they will be used in the following

The object of conservation *the* **genetic resource** consists of biological material which is either known to or for a *given* reason can be expected to contain either specific genetic information or a defined range of variants of such information In most cases the latter range is defined quantitatively but it may also include qualitative characterizations Hence the **declaration as genetic resource** of a given collection of biological material is dependent on the presence of genetic characteristics that might be of either a highly specific or an extensively variable nature In this respect **conservation** thus designates an activity that is directed towards the preservation of genetic material falling into either of the above categories of genetic variation

Sometimes the term gene conservation is applied in the general sense of conservation of genetic resources This is unfortunate in cases where for example a distinction has to be made between vegetatively and generatively maintained resources While in the first case the *genotype* is the unit of conservation in the second case the intermission of sexual processes imply recombination which leaves *genes* as the only possible units of conservation In this situation it might be advisable to distinguish between genotype and gene conservation

Turning to the present topic even though the precise objectives of conservation are quite numerous they can nevertheless be summarized under the following three categories

- Preservation of the potential for the realization of desired trait expressions (breeding products particular physiological adaptations etc)
- Preservation of adaptability of populations races or species (latent genetic potential resistance and tolerance factors etc)

- Preservation of unrecognized variation (population species and ecosystem protection)

These objectives are intimately related to the two major methods of conservation static and dynamic

Static conservation (obviation or minimization of micro- and macro-evolutionary processes) is particularly relevant in emergency situations where comparatively small or genetically highly specialized (uniform) collections are to be rescued from acute or predictable danger of extinction This includes genetic variants which can be expected to be of only temporary significance in production populations (resistance nutritional quality etc) For purposes other than those arising from immediate need this method of conservation has been criticized on various grounds For example losses during the conservation or intermittent and final regeneration phases of the statically conserved genetic resource may result in undesirably altered genetic compositions Among the most frequently cited causes are the accumulation of deleterious mutations or the evolution of adaptations to *in vitro* or *ex situ* conditions rather than to the relevant *in vivo* or *in situ* conditions These are strong arguments suggesting that statically conserved resources should again be exposed as soon as possible to the vagaries of evolutionary adaptation in order to avoid detachment of their genetic base from changing environmental demands

In contrast **dynamic conservation** explicitly allows for evolutionary processes and can therefore help to prevent most of the above problems Obviously dynamic conservation (*in* or *ex situ*) of a genetic resource requires protection of the resource population and in this way it also contributes to species protection Yet population protection cannot be achieved without inclusion of the supporting environment which thus necessitates ecosystem protection Two of the most basic measures determining the success of **population protection** are

- securing the (species-specific) demographic characteristics (habitat requirements population density subpopulation structure age class structure timing of sexual maturity etc) required for survival and reproduction and
- providing the genetic variation required for adaptation and preservation of adaptability

Consequently population protection and dynamic conservation of genetic resources have in common the objectives of securing the conditions for survival and reproduction and preserving the adaptability of populations Moreover genetic factors are likely to affect a considerable portion of the variation in demographic characteristics within species which further emphasizes the interdependence of protection

and conservation The insight that the persistence of a genetic resource is most effectively warranted by methods of dynamic conservation and that this in turn decisively depends on the possibility of population and ecosystem protection has only just started to attract attention in conservation philosophies and practice Remarkably this coincides with a period of increased awareness of ecological problems

Specific Concerns of Dynamic Conservation of Genetic Resources

There are however *demands* on dynamic conservation beyond those on population protection These demands are mostly *concerned* with the necessity of maintaining genetic variation in excess of that required for the preservation of adaptability As a rule such an excess constitutes a genetic load in the sense that the population fitness would increase after subtraction of individuals (and their successful gametes) carrying the excess genetic types Endangerment of resource persistence could be the consequence of an overly high genetic load Nevertheless under most systems of reproduction one may expect the genetic variants causing excessive load to be eliminated as a consequence of their selective disadvantage This however leaves the conservationist with the dilemma that dynamic conservation runs counter to her/his conservation efforts Therefore

> a major concern of dynamic conservation of genetic resources consists in specification of the conditions under which genetic variation existing in excess of that required for adaptation and constituting a genetic load can be maintained without endangering the adaptability of the resource population

As a rule the maintenance of genetic diversity requires sufficient **environmental heterogeneity** to allow for the evolution of differential adaptations However a distinction has to be made between spatial and temporal environmental heterogeneity since in combination with restricted gene flow the first offers the opportunity for the evolution of local adaptations with positive effects on the overall population fitness while the second implies adaptive lags that may lead to critical reductions in population fitness Thus a reduction of genetic load without loss of genetic diversity can be achieved by decreasing the temporal and increasing the spatially effective environmental heterogeneity Herewith spatially effective refers to that part of the local environmental variation that cannot be levelled out by migration or gene flow Random mating or unrestricted and undirected migration for example would effectively homogenize spatial environmental heterogeneity to a large extent Moreover equalization of the spatial representation of the

effective environmental heterogeneity may additionally increase the genetic diversity by increasing the genetic evenness

Another major factor that determines the success of a conservation measure is the **size of the resource population** Again any recommendation must proceed from the principles of population protection and must thus account for the basic *demographic* requirements for survival and reproduction of the species considered As far as the genetics of the resource are concerned a central problem is frequently seen in the selective forces that might reduce the genetic variation in the resource However as was shown above these forces may either be desirable in that they indicate advantageous adaptive evolution or they may be controlled by an appropriate apportionment of spatial and temporal environmental heterogeneity Thus the crucial factor consists in the amount of genetic drift taking place among selectively equivalent (neutral) genetic variants as a consequence of limited population size

Consequently from a genetic point of view

> the size of a resource population should be determined by the amount of genetic variation the loss of which by drift (at selectively neutral gene loci) is tolerable over a specified number of generations

Specification of the number of generations and the tolerable loss of variation are chiefly governed by practical reasoning The measurement of the amount of genetic variation should not be based on specific properties of genetic structures Heterozygosity for example is an inappropriate measure since depending on the mating system realized heterozygosity may vary almost independently of the numbers and *frequencies of* alleles at a gene locus More appropriate are measures of genetic diversity or differentiation

In summary since purely static conservation is efficiently applicable to a quite limited (though important) scope of objectives only dynamic conservation should be given considerably more room than it presently occupies in the field of conservation of genetic resources Moreover both the objectives and methods of conservation should be more explicitly oriented towards the preservation of adaptability which necessarily entails measures of population species and ecosystem protection

TAXONOMY OF CULTIVATED PLANTS - SOME EXPERIENCES FROM THE GATERSLEBEN GENEBANK

Karl HAMMER

The tasks of taxonomy with respect to cultivated plants were recently characterized as follows (HANELT 1988)

- to describe the often enormous variability by various methods and techniques in order to enable researchers of genetic resources to communicate on the representatives of this variability
- to relate this variability to ecological and geographical parameters
- to analyse and to explain the relationships between cultivated and closely related wild taxa in a (phylo)genetic context and
- to contribute to the understanding of the complex interactions between evolution and domestication (see HAMMER 1984) of cultivated plants and development and history of man

Characterization of the variability and the evaluation of evolutionary relationships as the two main contributions of taxonomy to the research of genetic resources are highly relevant to problems of applied botany mainly to the management of plant genetic resources in genebanks and to breeding aims (HANELT 1988)

HARLAN & De WET (1971) proposed a rational classification of cultivated plants which is rather useful for genebanks because it explains the genepool concept and presents an informal classification scheme on the infraspecific level The primary genepool is equivalent to the biological species Within this category two subspecies occur subspecies A which includes the cultivated races and subspecies B which comprises the spontaneous (wild and weedy) races (Fig 1)

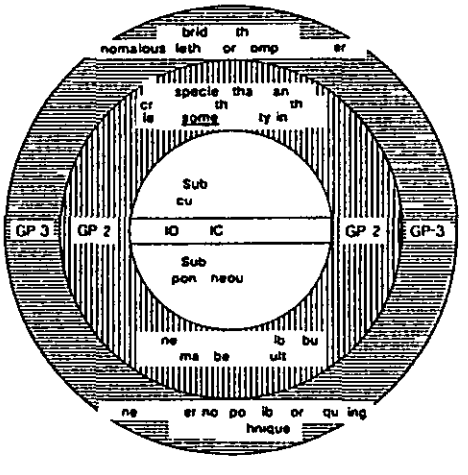


Figure 1

Classification of cultivated plants Schematic diagram from HARLAN & DE WET (1971)

GP-1 = primary genepool
GP-2 = secondary genepool
GP-3 = tertiary genepool

This simple classification prevents to a certain extent taxonomic studies on the infraspecific level The *Papaver somniferum* example (Table 1) shows that there are three subspecies which are geographically and/or evolutionary based (HAMMER 1981) As it has to be expected there is a growing artificiality in the lower levels of infraspecific classification For the distinction of botanical varieties e g seed- and flower colours are used At least for the seed colours a certain domestication trend can be pointed out As for *Papaver somniferum* for many other crops as well infraspecific systems have been elaborated at Gatersleben (see HAMMER 1981) By using these systems for classification genebank material can be well characterized and divided into morphologically defined lines This procedure is considered necessary to prevent losses during the reproduction of genebank material *ex situ*

Table 1 System of classifying *Papaver somniferum* proposed by DANERT (1958) modified by HAMMER (1981) see also HANELT & HAMMER (1987)

species level	*P p m ll m* L			
ssp level	ssp *m ll m*		ssp *g l m* BASIL	ssp *lg m* (DC) CORB
convar level	convar *m l fe m*	convar *l f ldll* HAMMER	convar *thm le l* HAMMER	convar *le t le* DANERT s. L
	indehiscent capsules	dehiscent capsules	indehiscent capsules	dehiscent capsules
var level	var *somnil m*	ar *dl oca p m* ALEF	var *lb s* VESS	var *l m* (ROTHM.) HAMMER
	ar *ndid m* VESS	ar *b p m m* VESS.	ar *blo nd m* VESS	var *p t m* DANERT
seeds white, yellowish, or pink	ar *oseol m* VESS	var *g l e m* VESS	var *hoda th m* VESS.	ar *f l m* DANERT
	var *p lll m* ALEF	ar *tll m* DANERT	var *lg um* DANERT	var *f lgid m* DANERT
	ar *ma o p m* COSS	ar *h tll* ALEF	var *pa m lat m* DANERT	var *m l m* DANERT
	ar *papy l m* DANERT	ar *plotill m* DANERT	var *aplat m* VESS.	var *h p l th m* DANERT
	ar *ola m* DANERT	ar *t m* DANERT	var *limb llo m* DANERT	ar *p ll l m* DANERT
seeds light grey light blue, or dark blue	var *h agea m* ALEF	ar *oo t stl m* DANERT	var *m d m* DANERT	var *le o m lum* DANERT
	var *o e l* ROTHM.	ar *p llid m* ROTHM.	ar *ll* VESS	var *ga s e* (ROTHM) DANERT
	var *oculatum* DANERT	var *m drll se* VESS	var *h l t m* DANERT	var *p aete t m* DANERT
	var *lg m* HAYNE	ar *q ssand m* ALEF	var *bid m* VESS.	var *t dil b m* DANERT
	ar *se e m* DANERT	var *pil th m* DANERT	var *sigill t m* DANERT	var *ocell t m* DANERT
	ar *bg lse m* VESS	var *b lol m* VESS.	ar *be l sum* DANERT	var *p lf m* DANERT

The elaboration of morphological classifications can also be done by using statistical methods e g principal component analyses (Fig 2) In this way the Cuban material of *Phaseolus lunatus* was classified (ESQUIVEL *et al* 1990) and the original taxonomic treatment of this crop by MACKIE (1943) could be supplemented Our findings are supported by evolutionary conclusions concerning the infraspecific groups

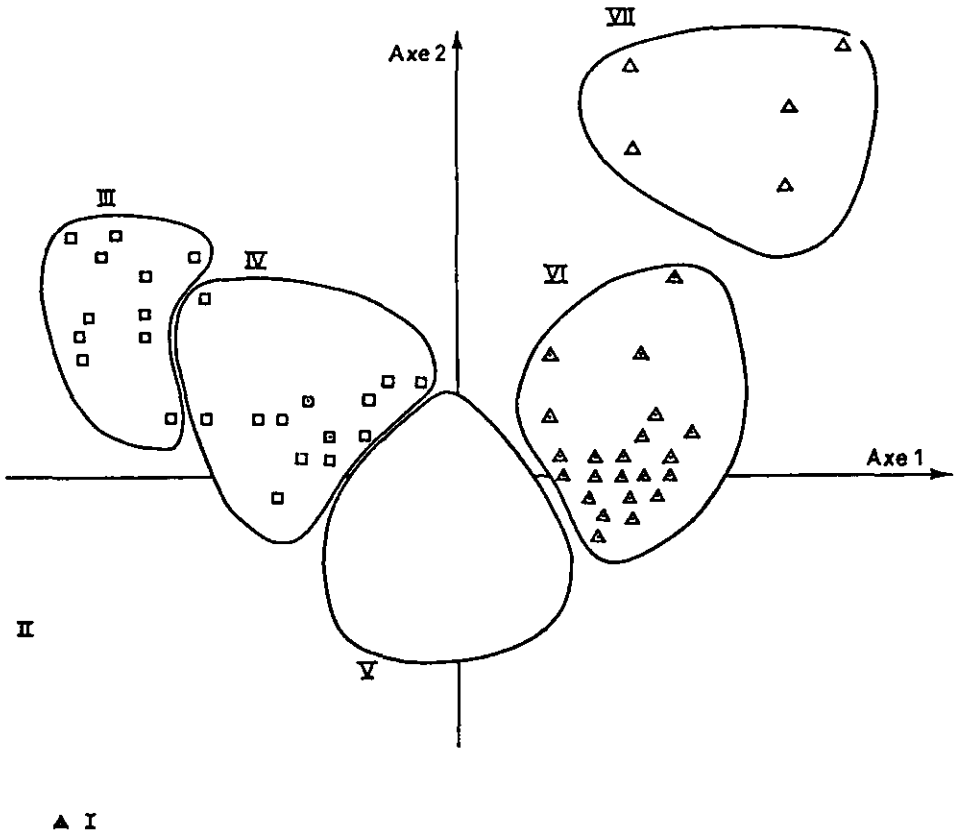


Figure 2 Result of the principal component analysis for *Phaseolus lunatus* from Cuba (from ESQUIVEL *et al* 1990)

I = wild
II = weedy
III = Potato
IV = Potato – Sieva
V = Sieva
VI = Sieva – Big Lima
VII = Big Lima

Knowledge about the breeding system is important for the reproduction *ex situ* In the genus *Secale* anther length turned out to be an important indicator of the breeding system (HAMMER *et al* 1987 HAMMER 1990) and allows a number of phylogenetic conclusions (Fig 3) These conclusions are the basis for the classification on the specific and infraspecific levels

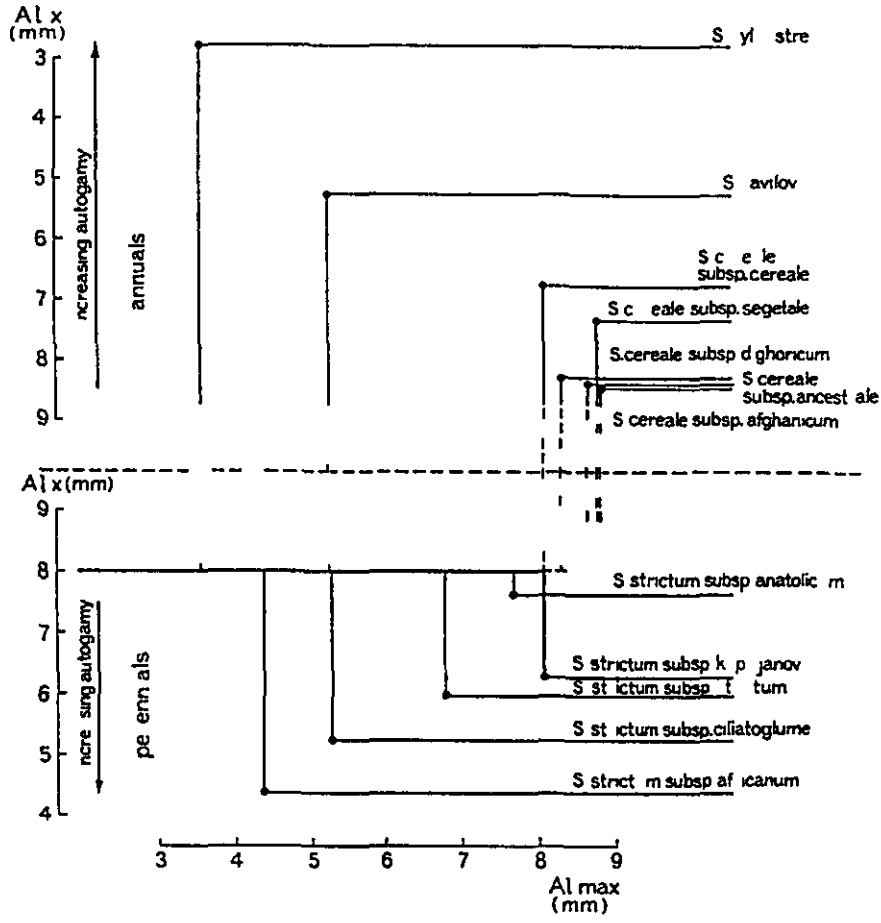


Figure 3 Phenogram constructed on the basis of anther characters for demonstrating evolutionary trends in *Secale* (after HAMMER 1990) Perennial and annual races are separated Delimitation of races according to HAMMER *et al* (1987)

Al max = maximum anther length
Al $\bar{x}$ = mean anther length

Though taxonomy is principally based on morphology other scientific fields have to be considered too and evaluated with respect to their usefulness for taxonomic conclusions Usually these conclusions can be drawn in cases in which it is possible to show evolutionary tendencies Within the genus *Aegilops* this could be demonstrated by using disease resistances against several pathogens (Table 2) The most primitive species *A speltoides* showed as a rule the highest resistance levels Also a geographical tendency could be demonstrated (HAMMER 1987)

Table 2 Disease resistances in the genus *Aegilops* (HAMMER 1987)

	Tilletia caries	*Ustilago tritici*	*Erysiphe graminis*	*Mayetiola destructor*	*Puccinia recondita*	*Puccinia striiformis*	*Schizaphis graminum*	*Puccinia graminis*	*Pseudocercosporella herpotrichoides*	*Septoria nodorum*	Genome
Ae mutica	100	71	100	0	0	33	–	0	0	0	M[1]
Ae speltoides	84	90	78	52	91	83	67	81	6	2	S
Ae longissima	100	29	96	50	23	23	75	6	0	0	S[1]
Ae searsii	–	36	–	–	67	35	–	0	0	–	S
Ae bicornis	86	0	83	40	8	0	0	0	33	0	S[b]
Ae peregrina	75	100	91	67	74	33	67	0	20	0	US
Ae kotschyi	50	29	100	0	13	20	0	0	20	0	US
Ae umbellulata	100	79	83	83	84	29	14	0	0	13	U[8]
Ae biuncialis	100	86	100	0	50	50	0	0	11	0	UM[b]
Ae columnaris	100	86	89	50	60	22	0	0	11	0	UM[c]
Ae neglecta	100	93	96	67	100	82	0	14	0	22	UM[1]
Ae geniculata	82	50	92	100	29	52	8	4	6	0	UM
Ae comosa	100	57	50	33	57	100	0	0	0	0	M
Ae uniaristata	100	93	0	–	0	75	–	25	0	50	M
Ae crassa	100	79	13	100	0	0	100	0	5	0	DM[a]
Ae ventricosa	90	86	75	100	0	46	0	0	29	0	DM
Ae juvenalis	100	100	67	–	0	0	–	0	0	0	DM[a]U
Ae tauschii	41	67	27	–	8	8	–	12	2	0	D
Ae cylindrica	100	43	54	100	35	0	0	0	0	15	CD
Ae markgrafii	83	29	71	50	71	80	100	60	0	0	C
Ae triuncialis	100	64	87	57	55	35	0	0	20	4	UC
Aegilops spp	84	70	66	60	40	33	18	14	6	4	

[1] after KRIVČENKO *et al* (1983)
[2] after NIELSEN (1985)
[3] combined from GILL *et al* (1985) and VALKOUN *et al* (1985)
[4] after GILL *et al* (1985)
[5] see VALKOUN *et al* (1985)
[6] see GROLL *et al* (1985)
[7] see FRAUENSTEIN & HAMMER (1985)
[8] see KIMBER & ABU BAKER (1981)

Table 3 Occurence of beet cryptic virus (BCV) in different beet races (from HAMMER *et al* 1990)

BCV 1 = beet cryptic virus 1 BCV 2 = beet cryptic virus 2

Material	BCV 1	BCV 2	Number of tests
***Beta vulgaris* var *rapacea* (fodder beet)**			
Altenburger Tonnen	+	+	1
Criewener Gelbe	+	+	2
Polyrouge	+	+	3
Rote Walze	+	+	4
Unikum	+	+	2
Žlta	–	–	1
***Beta vulgaris* var *vulgaris* (red beet)**			
Boliver Kogel	–	+	3
Boldurdy	–	+	2
Bordo	–	–	1
Carotine	–	–	1
Nina 4	–	–	1
Plattrunde Rote Feinlaubige	+	+	3
Podsi	–	+	3
Queen	–	+	2
Redpack	–	+	2
Renora	–	+	3
Rote Kugel	–	+	8 $
'Rubia	–	+	3
Ruddigore	–	+	3
Trianon	–	+	2
# K 5899 Soviet Union	+	+	3
# K 6871 Soviet Union	–	+	2
# K 7157 Italy	–	+	2
# K 7168 Italy	+	+	2
# Beta 155 Soviet Union	+	+	3
***Beta vulgaris* var *cicla* (chard)**			
Fudanso	+	–	4 $
Gruner Schnitt	–	–	1
Lucullus	+	–	7 $
# K 6596 Soviet Union	–	+	3
# K 6911 Italy	+	–	2
# K 7156 Iraq	+	+	3
# K 7187 Iraq	+	+	2
# K 7289 Italy	–	+	3

– leaf material from 5 to 10 different plants in mixture for each test
$ – material was tested also by ISEM-D
# – material from collecting missions

The differential occurrence of certain viruses can reflect evolutionary pathways too as in beets (HAMMER *et al* 1990) The results show that the genepools of chard and red beet have developed quite independently from each other (Table 3) Important parts of the taxonomic classification of beets (HAMMER 1986) could be supported in this way

This example already leads to the molecular level New methods in this field should be evaluated with respect to their usefulness for taxonomic purposes Within the genus *Hordeum* the fast and simple squash dot hybridization technique was used to determine redundancy differences in highly repeated DNA probes (JUNGHANS & HAMMER 1990) A large number of *Hordeum* races was screened using six different barley-specific highly repeated DNA probes (Table 4) Closely related races of *Hordeum vulgare* are characterized by a homogenous and high redundancy of repeated DNA tested As a consequence the races of the *Hordeum vulgare* complex should be considered as a single species In contrast wild barley species show different redundancies at a lower level Therefore this method is useful to elucidate phylogenetic tendencies especially on the species level Other methods e g RFLPs preferably apply to the infraspecific level

Table 4 Redundancy and distribution of genome-specific hrDNA probes in the genus *Hordeum* and some other cereals (after JUNGHANS & HAMMER 1990)

4 = 100 % of *H vulgare* 3 = 75 % of *H vulgare*
2 = 50 % of *H vulgare* 1 = 25 % of *H vulgare*

			pBHV28	pBHV107	pHB40 pHB96	pSH12 pSH89
Cultivated barley and closely related wild races						
H vulgare	convar *deficiens*	var *deficiens*	4	4	4	4
	convar *distichon*	var *medicum*	4	4	4	4
		var *nutans*	4	4	4	4
	convar *intermedium*	var *harlani*	4	4	4	4
	convar *labile*	var *steudelii-nigripallidum*	4	4	4	4
	convar *vulgare*	var *coelste*	4	4	4	4
		var *densum*	4	4	4	4
		var *dundarbeyi*	4	4	4	4
		var *himalayense*	4	4	4	4
		var *horsfordianum*	4	4	4	4

	pBHV28	pBHV107	pHB40 pHB96	pSH12 pSH89
H vulgare convar *vulgare* var *hybernum* 'Erfa	4	4	4	4
var *hybernum*	4	4	4	4
var *subparallelum*	4	4	4	4
H agriocrithon var *agriocrithon*	4	4	4	4
var *dawoense*	4	4	4	4
var *paradoxon*	4	4	4	4
H x lagunculiforme	4	4	4	4
H spontaneum x *H vulgare* convar *distichon*	4	4	4	4
H spontaneum var *bactrianum*	4	4	4	4
var *ischnatherum*	4	4	4	4
var *spontaneum*	4	4	4	4
var *transcaspicum*	4	4	4	4
Wild barleys				
H brachyantherum 2x	2	3	2	3
6x	3	4	3	1
H bogdanii	1	2	3	3
H brevisubulatum subsp *brevisubulatum*	1	3	2	1
subsp *turkestanicum*	2	3	2	1
subsp *violaceum*	1	2	3	1
H bulbosum subsp *bulbosum*	2	4	4	2
subsp *nodosum*	2	3	2	2
H capense	2	3	4	2
H chilense	1	3	1	2
H cordobense	1	2	1	2
H euclaston	1	1	2	3
H flexuosum	–	–	3	2
H intercedens	1	1	1	3
H jubatum	2	3	2	2
H lechleri	2	3	3	1
H marinum subsp *gussoneanum*	3	4	3	3
subsp *marinum*	3	4	3	3
H murinum subsp *leporinum*	3	3	2	2
subsp *murinum*	2	3	2	3
H parodii subsp *parodii*	2	3	3	3
subsp *santacrucense*	1	1	1	2
H procerum subsp *procerum*	2	2	2	2
subsp *setifolium*	1	1	1	3
H pubiflorum	2	2	–	–
H pusillum	1	1	2	1
H roshevitzii	2	3	2	2
H secalinum	2	3	–	3
Other species				
Avena sativa	1	0–1	1	0–1
Secale cereale Petka	0	0–1	–	–
Triticum aestivum Chinese Spring	0	0	0–1	0

Modern methods should be included into taxonomic approaches in cultivated plants But their usefulness has to be tested with respect to the plant group the taxonomic level within this group and other items of the plants under investigation This is a well-known fact from the work with morphological characters

For the taxonomy of wild species the specific level is most important whereas in cultivated species the infraspecific level plays an important role However in this plant group as well the specific level is still an object of taxonomy Nearly 5 000 species are cultivated (excluding ornamentals) on a world-wide scale (SCHULTZE-MOTEL 1986) To supplement and update this global list and as a useful tool for germplasm collecting area-specific checklists have been used (Table 5) (see also BAIK *et al* 1986 HAMMER *et al* 1988 ESQUIVEL *et al* 1989 and HAMMER *et al* 1990) The large amount of newly detected plants under cultivation in these regions stresses the actual importance of taxonomic investigations also on the species level

Table 5 Checklist of cultivated plants for areas

Area	Number of cultivated species	Number of collecting missions
Cuba	819	4
South Italy	520	10
Korea	515	5
Libya	279	3

References

BAIK M -C HOANG H -D & HAMMER K (1986) A check-list of the Korean cultivated plants Kulturpflanze 34 69–144

DANERT S (1958) Zur Systematik von *Papaver somniferum* L Kulturpflanze 6 61–88

ESQUIVEL M CASTIÑEIRAS L KNÜPFFER H & HAMMER K (1989) A checklist of the cultivated plants of Cuba Kulturpflanze 37 211–257

ESQUIVEL M CASTIÑEIRAS L & HAMMER K (1990) Origin classification variation and distribution of lima bean (*Phaseolus lunatus* L) in the light of Cuban material Euphytica 48 1–9

FRAUENSTEIN K & HAMMER K (1985) Prufung von *Aegilops*-Arten auf Resistenz gegen Echten Mehltau *Erysiphe graminis* DC Braunrost *Puccinia recondita* ROB ex DESM und Spelzenbraune *Septoria nodorum* BERK Kulturpflanze 33 155–163

GILL B S SHARMA H C RAUPP W J BROWDER L E HATCHETT J H HARVEY T L MOSEMANN J G & WAINES J G (1985) Evaluation of *Aegilops* species for resistance to wheat powdery mildew wheat leaf rust Hessian fly and green-bug Plant Disease 69 314–316

GROLL U FRAUENSTEIN K & HAMMER K (1985) Prufung von *Aegilops*-Arten auf Resistenz gegen *Pseudocercosporella herpotrichoides* (FRON) DEIGHTON Kulturpflanze 33 165–172

HAMMER K (1981) Problems of *Papaver somniferum* – classification and some remarks on recently collected European poppy landraces Kulturpflanze 29 287–296

HAMMER K (1984) Das Domestikationssyndrom Kulturpflanze 32 11–34

HAMMER K (1986) *Beta* L In Rudolf Mansfelds Verzeichnis landwirtschaftlicher und gartnerischer Kulturpflanzen (ohne Zierpflanzen) ed by SCHULTZE-MOTEL J 145–151

HAMMER K (1987) Resistenzmerkmale und Reproduktionssystem als Indikatoren fur evolutionare Tendenzen in der Gattung *Aegilops* L Biol Zentralbl 106 273–282

HAMMER K (1990) Breeding system and phylogenetic relationships in *Secale* L Biol Zentralbl 109 45–50

HAMMER K KNÜPFFER H & PERRINO P (1990) A checklist of the South Italian cultivated plants Kulturpflanze 38 (in press)

HAMMER K LEHMANN C O & PERRINO P (1988) A checklist of the Libyan cultivated plants including an inventory of the germplasm collected in the years 1981 1982 and 1983 Kulturpflanze 36 475–527

HAMMER K SKOLIMOWSKA E & KNÜPFFER H (1987) Vorarbeiten zur monographischen Darstellung von Wildpflanzensortimenten *Secale* L Kulturpflanze 35 135–177

HAMMER K STANARIUS A & KÜHNE T (1990) Differential occurrence of beet cryptic viruses – a new tool for germplasm characterization and evolutionary studies in beets? Euphytica 45 23–27

HANELT P (1988) Taxonomy as a tool for studying plant genetic resources Kulturpflanze 36 169–187

HANELT P & HAMMER K (1987) Einige infraspezifiche Umkombinationen und Neubeschreibungen bei Kultursippen von *Brassica* L und *Papaver* L Feddes Rep 98 553–555

HARLAN J R & DE WET J M J (1971) Toward a rational classification of cultivated plants Taxon 20 509–517

JUNGHANS H & HAMMER K (1990) Sind Redundanzunterschiede hochrepetitiver DNA fur phylogenetische Schlußfolgerungen in der Gattung *Hordeum* geeignet? Biol Zentralbl (in press)

KIMBER G & ABU BAKER M (1981) The genomic relationships of *T dichasians* and *T umbellulatum* Z Pflanzenzuchtg 265–273
KRIVČENKO F I JAMALEEV A M ISAEV R F & MIGUŠOVA É F (1983) Ustojčivost ègilopsa k tverdoj golovne Bjul VIR 129 18–21
MACKIE W W (1943) Origin dispersal and variability of the Lima bean (*Phaseolus lunatus* L) Hilgardia 15 1–29
NIELSEN J (1985) *Ustilago* spp pathogenic on *Aegilops* II *Ustilago tritici* Can J Bot 63 765–771
SCHULTZE-MOTEL J (ed) (1986) Rudolf Mansfelds Verzeichnis landwirtschaftlicher und gartnerischer Kulturpflanzen (ohne Zierpflanzen) 4 Vols
VALKOUN J HAMMER K KUČEROVÁ D & BARTOŠ P (1985) Disease resistance in the genus *Aegilops* L – stem rust leaf rust stripe rust and powdery mildew Kulturpflanze 33 133–153

GENETIC VARIATION IN CROP SPECIES AND THEIR WILD RELATIVES

Daniel G DEBOUCK – Report of Working Group A

Participants
B BECKER D G DEBOUCK E DETTWEILER H-R GREGORIUS
K HAMMER A McCUSKER G MIX-WAGNER H-P MÜLLER

The working group expressed its interest in seeing more studies carried out on the patterns of plant genetic diversity in space and time particularly in view of the balance of diversity present inside natural populations and between them The group recommended that the following biological and genetic aspects should deserve further in-depth study through projects on plants of mutual interest

- the differential seed production between individuals giving opportunity for certain cytoplasmic information to become dominant in a population (extension of studies carried out on *Vicia* monitoring in the wild for other selected crop species and refinement of appropriate molecular markers)

- the differences in maturity in the gametophytes during the course of a season especially for certain tree species and their possible consequences for the diversity in the next generation (particularly in view of a sampling done for germplasm purposes at different times)

- the causes of those differences (stresses competition inside population consequences of indiv dual physiological reactions etc)

In case these causes involve any climatic factors the group was interested in measuring the variation from one year to another Perhaps in parallel to those studies to be carried out in natural populations the group suggested similar studies be carried out on samples of populations of the same plant material in order to better control the genetical drift during management practices in germplasm collections *ex situ*

Finally particular interest was expressed by one participant to see more work carried out on the Asian genepool of the genus *Vitis* for both purposes of germplasm conservation and enhancement

POTENTIAL AND LIMITATIONS OF CURRENT METHODOLOGIES FOR INVESTIGATING GENETIC DIVERSITY

Toby HODGKIN – Report of Working Group B

Participants
A –W ALFERMANN G FISCHBECK T HODGKIN H –J JACOBSEN G KAHL L SCHILDE-RENTSCHLER U SEITZ

The working group agreed to concentrate its discussions on the potential application of molecular genetic techniques in the study of plant genetic diversity

The direct practical experience of two participants of the workshop G KAHL and G FISCHBECK in relevant molecular genetic research was noted as was the experience of a number of other German scientists in this area

The working group identified a number of important objectives for genetic diversity research using molecular genetic techniques

- evaluating the potential of techniques such as DNA finger-printing in the identification of duplicate accessions in genebanks
- comparing genetic diversity analyses using molecular genetic markers with results obtained using morphological or biochemical data
- evaluating the potential of molecular genetic markers in screening germplasm collections for desirable characters such as disease resistance
- describing variation in land races at the molecular genetic level as an aid to the production of crop varieties suited to sustainable agriculture
- developing molecular genetic techniques more suited to use in developing countries (e g avoiding the use of radiolabelled probes) and the provision of training for personnel from developing countries

The working group was convinced that there were immediate opportunities for work using molecular genetic techniques in barley chickpea lentil and yam and that at a later date work on minor Andean tubers would also be valuable Although there are a number of crop-specific objectives it is likely that suitably planned research projects would deal with a large number of the objectives listed above

PROBLEMS AND METHODOLOGIES FOR MANAGEMENT AND RETENTION OF GENETIC DIVERSITY IN GERMPLASM COLLECTIONS

V Ramanatha RAO – Report of Working Group C

Participants
Chr LEHMANN U von POSCHINGER-CAMPHAUSEN V R RAO S SCHITTENHELM

The working group considered the problems as discussed during the presentation of V R RAO on regenerating seed of germplasm The major issues identified by the group were

- effect of mutation rate and direction of mutation
- isolation requirements/methods
- pollination control methods
- relationship between actual population size and effective population size
- effect of the variation in gametic contribution on effective population size
- estimation of genetic erosion in gene banks over regenerations
- crossing techniques for regeneration of germplasm
- *reproductive biology and breeding systems*

After careful consideration of interests of both IBPGR and German scientists the following items were identified that could be developed into projects to provide information necessary to develop appropriate strategies to regenerate outbreeding crops

Effective pollination control methods in the regeneration of germplasm

This includes identifying cost effective methods to promote random outcrossing in crosspollinated crops with emphasis on identifying the most effective pollinator Insect behaviour during pollination breeding and multiplication patterns will also be studied Another important issue is the effect of screening/cages/bags on seed quality Different population sizes will be included to determine the most appropriate number of plants to be grown and the most adequate pollination control methods to be used

This work is essentially viewed as a joint effort between FAL (Braunschweig) and ZiGuK (Gatersleben) IBPGR will help in planning the project and in disseminating the results which are expected to be widely applicable in genebanks in both developing and developed countries

Comparative studies on the efficiency and cost effectiveness of crossing techniques in the regeneration of allogamous crop germplasm

Various available crossing techniques e g pair crossing polycross etc will be tested using different population sizes These tests will provide information needed for regeneration of allogamous species Markers (enzymic molecular) could be used to clearly follow the contribution of individuals to genetic variability in the next generation Such research could be expanded to provide opportunities for collaboration with various interested national programmes

SPECIFIC PROJECT PROPOSALS ANNEX 1

At a two-day workshop discussions were held between scientists representing the Federal Republic of Germany the German Democratic Republic and IBPGR to explore areas of common interest in research on genetic resources of crop genepools In particular discussions focused on crop conservation strategies for roots and tubers and on genetic diversity studies

A list of six potential collaborative projects was compiled during the workshop Two of three projects proposed by IBPGR were identified as being of particular interest for further development and short-term implementation 1) Refinement of Cryopreservation Techniques for Potato and 2) Spatial and Temporal Distribution of Genetic Diversity in Wild Forage Species under Stress Conditions

Refinement of Cryopreservation Techniques for Potato

IBPGR regards the refinement of cryopreservation technologies as a most important step in conserving the germplasm of root and tuber crops In this context potato is being considered as the most promising species

Success in freezing potato cultures in liquid nitrogen and subsequently thawing and re-establishing them has already been achieved in several laboratories Since it has not yet been possible to achieve success with sufficient frequency to permit the use of cryopreservation as an appropriate technology for germplasm conservation the project should contribute to developing this technology towards a more reliable routine method

L A WITHERS IBPGR has agreed to participate in the planning and coordination of the project A number of German scientists are also interested in research collaboration Technical support access to *in vitro* culture- and cryopreservation equipment and access to appropriate germplasm would be necessary

Spatial and Temporal Distribution of Genetic Diversity in Wild Forage Species under Stress Conditions

The aim of this project would be to develop and apply methods for sampling the genetic diversity of selected Sahelian forage species growing over a wide area but possibly separated into distinct genepools

isolated from each other by space and/or flowering time This investigation would serve as the basis for designing appropriate germplasm collection and conservation methods for plant populations growing under stress conditions and flowering in response to intermittent rainfall rather than in a regular seasonal pattern

Target species will be chosen according to their importance in local agricultural systems and practices their potential use in environment rehabilitation and their suitability as models for wider application

It is envisaged that the project would engage a German post-doctoral fellow who would have the major responsibility for the day-to-day scientific programme and an IBPGR Research Associate preferably recruited from a country in the region These two persons would constitute the survey team and would both be based at Niamey Niger

IBPGR s input to the supervision of the project would be made by the Research Officer (Wild Species) based in Rome and the Coordinator for West Africa based in Niamey

A third project **Effective Pollination Control Methods in the Regeneration of Cross-Pollinated Crops** was regarded as being of particular scientific importance at this time Its results would be widely applicable to the multiplication and regeneration of genebank material in developing and developed countries

This project would most logically be jointly carried out by the Institute of Crop Science and Plant Breeding at the FAL in Braunschweig and the Institute of Genetics and Crop Plant Research (ZIGuK) at Gatersleben Although there is no justification for IBPGR s formal involvement in the project administration IBPGR would be prepared in assisting with the project design and in disseminating the experiences and results this includes the eventual transfer of the methodology to genebanks in developing countries

Another three projects which are however not ready for immediate implementation were identified as being of great interest to both German scientists and IBPGR

- **Molecular genetic diversity in barley land races from South-West Asia**
- **The development of a strategy for the conservation of genetic diversity in West African yams**
- **Comparative studies on efficiency and cost effectiveness of crossing techniques in the regeneration of allogamous crop germplasm**

WORKSHOP PROGRAMME ANNEX 2

Sunday 6 May 1990

20 00 h Dinner at Bonner Stuben Wilhelmstrasse 22 Bonn

Monday 7 May 1990

Opening Session
(Chairperson H WELTZIEN)

8 30 h Opening Remarks (H WELTZIEN)

8 40 h A Few Remarks on the Agricultural Research Aid Focus (H –J DE HAAS)

8 50 h
- The Plant Genetic Resources Approach of the Federal Republic of Germany (G MIX–WAGNER as a substitute for M DAMBROTH)
- Programme Presentation (H WELTZIEN)

9 00 h IBPGR s Research Programme (A McCUSKER)

9 20 h Presentation of Participants (H WELTZIEN)

11 00 h Coffee Break

Part I Crop Strategies for Roots and Tubers
(Chairperson H –J JACOBSEN)

11 20 h Crop Strategies for Roots and Tubers Potato – a Model for Refinement Yam – a Problem for Development (L A WITHERS)

11 50 h Discussion

12 30 h Lunch

14 00 h Contributions by German Scientists
(presentations 15 min discussions 5 min)
- Fundamental Aspects of Plant Regeneration (H –J JACOBSEN)

- *In Vitro* Propagation of Yam and Perspectives for its Long-Term Conservation (G MIX-WAGNER)
- Protoplast Fusion as a Technique in Breeding of Potato and Other Tuber Crops (L SCHILDE-RENTSCHLER)
- Cryostorage of Plant Material (U SEITZ)

15 30 h Coffee Break

15 50 h Plenary Discussion Crop Strategies for Roots and Tubers (L A WITHERS)

20 00 h Dinner at MAREDO Wesselstrasse 5 Bonn

Tuesday 8 May 1990

Part II Genetic Diversity
(Chairperson G FISCHBECK)

8 30 h *Introduction* (A McCUSKER)

8 40 h Introduction (G FISCHBECK)

8 50 h Genetic Variation in Crop Species and Their Wild Relatives A Viewpoint for Their Conservation (D G DEBOUCK)

9 05 h Potential and Limitations of Current Methodologies for Investigating Genetic Diversity (T HODGKIN)

9 20 h Problems and Methodologies for Management and Retention of Genetic Diversity in Germplasm Collections (V R RAO)

9 35 h Discussion

11 00 h Coffee Break

11 20 h Contributions by German Scientists
(presentations 15 min discussions 5 min)

- Characterization of Genetic Diversity within Core Collections by Restriction Fragment Length Polymorphisms (RFLP) (G FISCHBECK)
- Changing Priorities in Conservation Objectives of Genetic Resources (H -R GREGORIUS)
- Taxonomy of Cultivated Plants - Some Experiences from the Gatersleben Genebank (K HAMMER)

12 30 h	Lunch
14 00 h	Work ng Groups
	A Genetic Variation in Crop Species and Their Wild Relatives (D G DEBOUCK)
	B Potential and Limitations of Current Methodologies for Investigating Genetic Diversity (T HODGKIN)
	C Problems and Methodologies for Management and Retention of Genetic Diversity in Germplasm Collections (V R RAO)
16 00 h	Coffee Break
16 30 h	Results and Recommendations of the Working Groups
17 30 h	Closing Remarks (G FISCHBECK)

Wednesday 9 May 1990

Excursions of the IBPGR Scientists

7 30 h	Visit to the Max-Planck-Institute for Resistance Breeding Cologne
14 00 h	Visit to the Institute for Genetics University of Bonn

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BML	Bundesministerium fur Ernahrung Landwirtschaft und Forsten (Federal Ministry of Food Agriculture and Forestry) Bonn Federal Republic of Germany
BMZ	Bundesministerium fur Wirtschaftliche Zusammenarbeit (Federal Ministry for Economic Cooperation) Bonn Federal Republic of Germany
CGIAR	Consultative Group on International Agricultural Research Washington D C USA
CIAT	Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture) Cali Colombia
CIP	Centro Internacional de la Papa (International Potato Center) Lima Peru
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (*German Collection* of Microorganisms and Cell Cultures) Braunschweig Federal Republic of Germany
FAL	Bundesforschungsanstalt fur Landwirtschaft (Federal Research Centre for Agriculture) Braunschweig-Volkenrode Federal Republic of Germany
FAO	Food and Agriculture Organization of the United Nations Rome Italy
IBPGR	International Board for Plant Genetic Resources Rome Italy
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics Hyderabad India
IITA	International Institute of Tropical Agriculture Ibadan Nigeria
IUCN	The World Conservation Union Geneva Switzerland
ZIGUK	Zentralinstitut fur Genetik und Kulturpflanzenzuchtung (Institute of Genetics and Crop Plant Research) Gatersleben then German Democratic Republic