EUROPEAN COOPERATIVE PROGRAMME FOR THE CONSERVATION AND EXCHANGE OF CROP GENETIC RESOURCES

IBPGR

REPORT OF A WORKING GROUP ON FORAGES

(third meeting) held at the Station de Génétique et d'Amélioration des Plantes de l'INRA Mauguio, Montpellier, France 10-12 January 1989

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

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EUROPEAN COOPERATIVE PROGRAMME
FOR THE CONSERVATION AND EXCHANGE OF CROP GENETIC RESOURCES
(ECP/GR)

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ECP/GR/IBPGR
Rome, 1989

The International Board for Plant Genetic Resources (IBPGR) is an autonomous international scientific organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR). IBPGR was established by 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 Environment Programme and the World Bank.

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INTRODUCTION

The third meeting of the ECP/GR Forage Working Group was held, 10-12 January 1989, at the Station de Génétique et d'Amélioration des Plantes de l'Institut National de la Recherche Agronomique (INRA), in Mauguio, near Montpellier, at the invitation of its Director, Dr. P. Mansat.

Dr. Mansat and Dr. A. Charrier (Director of the French Bureau des Ressources Génétiques) welcomed the participants and both presented the work of their institutes. The meeting noted the regrets of Dr. Abd Moneim, representative of ICARDA, who was unable to attend. A list of participants is provided in Appendix I. Mr. B.F. Tyler was unanimously elected as Chairman. The Agenda, as approved, is shown in Appendix II. Participants had an opportunity to visit the Station de Mauguio and the ORSTOM Centre in Montpellier, including the Laboratory on Genetic Resources and Breeding for Tropical Plants, on the morning of 12 February.

REPORT

REVIEW OF CURRENT ACTIVITIES

European Forage Data Bases

1. In 1987 the Plant Breeding and Acclimatization Institute (IHAR), Radzikow, Poland, published a second edition of the European catalogue for Festuca and one for Dactylis, and the Welsh Plant Breeding Station (WPBS), Aberystwyth, UK, published European catalogues for Lolium perenne, Lolium multiflorum and Trifolium repens. The Institute for Crop Science and Plant Breeding, FAL, Braunschweig, FRG, produced, in October 1988, the first edition of the European Poa catalogue, which was distributed at the meeting. Dr. P. Guy, on behalf of INRA-GEVES, La Minière, France, presented the third edition of the European catalogue of Medicago (perennial species) (September, 1988) and Dr. S. Badoux, from the Federal Agricultural Station of Changins, Switzerland, presented the European catalogue of Trifolium pratense (second edition, December 1988). Dr. M. Martín Bellido, from Servicio de Investigación Agraria de Extremadura, Badajoz, Spain, presented the ECP/GR Officer at the meeting with a copy of the European catalogues for Trifolium subterraneum and Medicago (annual species, second edition, December 1988). These last two catalogues will be widely distributed. All the catalogues mentioned above follow the standardized European format agreed on by European Forage Data Bases and their producers were congratulated.

   Near completion are the catalogues on perennial Lathyrus from the University of Pau, Pau, France; on Vicia and annual Lolium, from the Germplasm Institute, Bari, Italy; and Bromus, from the Research Centre for Agrobotany (RCA), Tapioszele, Hungary. Dr. B. Cagas, from the Grassland Research Station, Roznov, Czechoslovakia, presented draft European catalogues on Trisetum flavescens and Arrhenatherum elatius and requested the active collaboration of holders of these two species to send data on their accessions. Dr. P. Perrino, of the Germplasm Institute, Bari, asked for collaboration of curators holding Hedysarum and Phalaris so that comprehensive catalogues may be published. The University of Southampton is building up a European Forage Data Base for Lathyrus spp., separate from that produced by the University of Pau, and the first editions of these two catalogues are expected in the near future. A list of institutes acting as European Forage Data Bases is provided in Appendix III.

Members noted that the European forage catalogues had intrinsic publicity value for the collections in question and were also useful for informing agricultural decision-makers about work in progress.

2. The session of the Forage Working Group held at INRA, Lusignan, 24 September 1987, had recommended that each national genebank/major forage institute document the collected accessions from that particular country (or collected by them) for the descriptors "latitude", "longitude" and "altitude", without which database files would have limited value. Members reported on achievements and future activities regarding this topic. Exchange of data in the form of diskettes has been increasing over the last year.

Mapping of collected accessions

3. The second meeting, held at Oak Park Research Centre, Carlow, Ireland, October 1985, had recommended that data accumulated in databases should be used to prepare maps of original locations of samples in order to identify gaps in collections. Members requested IBPGR to help to identify suitable computer programs to do this job.

Progress in collecting

4. The session of the Forage Working Group in Lusignan had already reported on progress in collecting after the recommendations of the second meeting of the ECP/GR Forage Working Group, as follows: red clover had been collected in Belgium by the Government Plant Breeding Station, Merelbeke, Belgium, in Greece by the Fodder Crops and Pastures Institute (FCPI), Larissa, Greece, and in the Netherlands by the Centre for Genetic Resources, the Netherlands, Wageningen; white clover had been collected in Greece by FCPI; lucerne and annual medics in Spain and south Portugal by INRA, France and ICARDA; Lolium perenne in Austria, FRG (Bavaria and Upper Rhine) and the UK (Yorkshire) by WPBS, UK; Lolium multiflorum in Italy by the Forage Crop Research Institute, Lodi, Italy; and Hedysarum coronarium had been collected in Tunisia, Malta, Italy (Sardinia) and Morocco by various institutes.

The following information was provided by participants:

Some collecting for Lathyrus and Vicia spp. had been conducted by the University of Southampton, Southampton, UK, in the UK, France and Spain. The same institute participated in an USDA-IBPGR collecting mission to northwest Yugoslavia for Vicia, Lathyrus, Medicago and endemic Trifolium species. RCA, Hungary, had collected a range of 122 accessions of forages in continuation of their routine programme. The National Plant Breeding Station, Elvas, Portugal, had collected Ornythopus and Trifolium spp. in southern areas of the country. WPBS had collected Lolium perenne and Trifolium repens from endangered habitats in the UK and had cooperated with the University College of Aberystwyth for collecting diploid forms of Festuca pratensis in Sweden. In the COMECON countries, meadow, fescue, tall oat grass and yellow oat grass, ryegrass and tall fescues had been collected in 1987 in Slovakia, Czechoslovakia, and a similar group of species had been collected in GDR in 1988. In 1987, IHAR, Radzikow, Poland, had collected 600 accessions of grasses in the southern region of Poland and 27 Trifolium medium accessions. While no specific collection of forages had been undertaken by the Germplasm Institute, Bari, Italy, some opportunistic collecting had taken place in Italy.

IBPGR had supported a collecting mission of Vicia, Lathyrus and forage legumes in Syria in 1986 and southwest Turkey in 1987. A major expedition to collect forage legumes in southeast Turkey had taken place in 1988 in a region to be totally flooded with water due to the construction of the Ataturk dam. Participants requested information on the dissemination of seeds collected under IBPGR auspices and sought assurances that these resources were destined for the appropriate genebanks. The IBPGR collecting officer provided this information.

List of standard varieties

5. A list of standard varieties of forage legumes in Mediterranean zones for use as reference when evaluating genetic resources material had been finalized during the working session in Lusignan. Members noted with satisfaction that AGRIMED had recommended the use of these standard varieties for genetic resources.

6. The meeting in Lusignan had also finalized the list of reference varieties for forage genetic resources in northern and middle Europe. The working session had agreed that curators of European Data Bases should each hold a large stock of these seeds, for the species under their responsibility, for immediate distribution. It was noted that institutes acting as databases had obtained 2 to 5 kg of seed of nearly all the reference varieties. Members requested the ECP/GR Secretariat to continue to help these institutes obtain the few remaining reference varieties not yet in stock.

   The interest raised outside Europe by these lists of standard varieties was mentioned. Members expressed their appreciation to Dr. P. Guy, who, as coordinator for these lists, played a key role.

IBPGR progress on the survey of forage genetic resources in the Mediterranean area

7. The first stage of a survey by IBPGR on the forage genetic resources in the Mediterranean area (collation of data on 21 400 accessions in a database) had been reported at the working session in Lusignan. The IBPGR collecting officer informed the meeting that a second stage is now under way. A scientist funded by IBPGR and stationed at ICARDA, Syria, will update the geographical coordinates of each accession to facilitate the accurate description of the distribution of individual species. Mr. R. Reid reported ICARDA's wishes to establish closer working links with the European Forage Data Bases and the ECP/GR Forage Working Group. The meeting was in favour of such collaboration (see para. 22).

International network on medics

8. The members were informed of IBPGR's intention to support the implementation of international crop genetic resources networks, the genus Medicago, as one of the major taxa, having been selected for a pilot project. A meeting on the use of annual medics in Mediterranean ley farming will be convened by ICARDA and the University of Perugia, Italy. At this meeting and with the support of IBPGR, delegates will discuss the initiation of the proposed Medicago network. French Medicago specialists expressed their intention to bring suggestions and contribute in other ways towards the implementation of such a network.

Strategies for maintenance of forage genetic resources

9. Seven discussion papers dealing with maintenance/enhancement of forage genetic resources were presented at the meeting. These are reproduced in Appendix V. Each of these papers was well received. During the in-depth discussion that followed, numerous conclusions were reached. These are summarized in the workplan.

WORKPLAN

European Forage Data Bases

10. A format for recording genetic resources data for exchange (see Appendix IV, which is basically the one recommended by the Workshop on Exchange of Information, Radzikow, 1984, was presented by Dr. Serwinski. The systematic use of this format was strongly recommended by participants. Furthermore, members agreed that this format be recommended to other organizations exchanging plant genetic resources data (e.g. IUCN).

11. Any exchange between curators and those responsible for databases should be on floppy disks or magnetic tape. Having recognized the publicity value of catalogues the meeting stressed that data files should also be distributed in the form of diskettes to facilitate the orderly use of the data. The meeting agreed that diskettes of the databases be routinely forwarded to IBPGR Headquarters where transfer support or other services can be given on an ad hoc basis.

12. To date, catalogues had been distributed mainly to data contributors and country coordinators. There was general consensus that other interested parties such as universities, breeders' groups etc. should be reached and that individual members of the Working Group and ECP/GR/IBPGR should publicize the existence of these databases.

    Members agreed that a booklet summarizing the information in the databases would be compiled by ECP/GR/IBPGR in the near future and widely distributed. This booklet was the best way to publicize the ECP/GR Forage Working Group and would aid the successful coordination of its activities.

Regeneration and core collections

13. A lengthy discussion took place on regeneration problems and the consensus reached was that financial constraints were the major limiting factor. It was noted that in a polycross of an outbreeding grass population there could be bias toward genotypes with high seed-producing capacity at the expense of the lower seed producers, which are often the more valuable pasture types. The meeting recommended that seed of the mother plants of the original multiplication be stored separately and used for subsequent regeneration. It was further stressed that regeneration outside the region of origin, when control of the regeneration/environment was not possible, could result in selective elimination or non-contribution of genotypes and thus genetic shift. The meeting showed considerable interest in the "backyard" regeneration that had been performed by selected farmers in Hungary. S. Horvath reported that last year approximately 100 populations were regenerated in this way.

14. In view of the real problems associated with the regeneration of entire collections, the meeting considered the possibility of establishing core collections. A core collection was defined by the Working Group as a subset of accessions that represents as far as possible the existing variability of all the accessions. There was a general consensus that such core collections should not be established within an institute but across all institutes holding the same genera/species. Consequently, the meeting recommended that a pilot study be implemented within the ECP/GR. This study would enable positive decisions to be made regarding the establishment of core collections and provide guidelines for implementation. Members agreed that such a project would be a significant step in strengthening the European network. It was further agreed that a draft proposal be submitted to the TCC of the ECP/GR in October 1989. Members nominated a team to draft this proposal consisting of B.F. Tyler, WPBS (grass specialist), a French expert not yet designated (methodology) and another expert on legumes (an Italian expert was suggested). It was requested that IBPGR provide technical support to the formulation of this project.

15. The meeting congratulated IBPGR on the past quality of its technical manuals and, in view of this, it was requested that IBPGR consider the production of a practical manual dealing with the regeneration of temperate forage species.

In situ conservation

16. The Group discussed the concept of in situ conservation in the context of European conservation areas. It was agreed that in situ conservation was extremely valuable and complementary to established systems of ex situ conservation. The Swedish achievements in establishing a system of in situ conservation, which includes forage species, stimulated great interest (see Appendix V). Members recommended that studies be undertaken to follow up the evolution of genotypes under this unique system which would, it was hoped, serve as a model for other countries. The meeting further highlighted the effects of management systems on the maintenance of genotypes. It was recognized that only national and international conservation organizations are effectively able to designate areas of biological significance for conservation purposes. However, it was strongly recommended that the forage community make its priorities and requirements known to the relevant conservation organization. This can be stimulated through the implementation of a pilot study that can identify i) species, ii) location, iii) intrinsic value and iv) management systems. All members were ready to cooperate with this study but recognized that the coordinating role had to be undertaken by an organization such as IBPGR.

Duplication of accessions for safety

17. The meeting noted that, with the exception of Kew, with its global responsibility for Trifolium species, there were no designated base collections[1/] for temperate forages. A lengthy discussion followed on the necessity to identify base collections for temperate forages. It was unanimously accepted that at least for the time being, this concept of base collection was too constraining for temperate forage curators. Nevertheless, the need for a systematic safety duplication was stressed.

1/ Base collections, as defined by IBPGR, are intended for long-term seed storage at -10 to -20°C, and not for exchange of material unless such material is unavailable in active collections. Arrangements for regeneration should be made with the active collections holding the accession when viability falls below an accepted level.

Within the European context members agreed to build up an informal system through which curators of databases would identify relevant accessions of the species under their responsibility held in only one collection; each curator would endeavour to duplicate these accessions in another forage collection that had long-term storage facilities.

Standard varieties

18. The meeting recommended that a technical bulletin listing the reference varieties for northern and middle Europe and the addresses of the curators holding these varieties be published as soon as possible and distributed as widely as possible, including organizations such as the EEC, OECD, COMECON, etc.

19. The Group recommended that the same system be used for reference varieties of forage legumes in the Mediterranean, i.e. a large quantity of seeds of each reference variety should be held by the person responsible/curator for a database for immediate distribution to those who request it. The ECP/GR Secretariat was requested to initiate action. Members also recommended that the list for the Mediterranean zone be completed with reference varieties for grasses.

Further collecting

20. Further collecting is planned by the University of Southampton in 1989 for a broad range of forage legumes in the Caucasus region of the USSR. The meeting was also informed that Czechoslovakia intended to collect forages in the same region. The National Plant Breeding Station, Elvas, Portugal, intends to collect Ornythopus and Trifolium species mainly in the north of the country and it is hoped that in the future systematic collections will be undertaken in the Azores with IBPGR support. Servicio de Investigación Agraria (SIA) of Extremadura, Badajoz, will collect Medicago spp., Ornythopus spp. and Trifolium spp. in southeast Spain and Portugal in 1989. The Forage Crop Research Institute, Lodi, Italy, is presently collecting Trifolium repens in northern Italy. INRA, France, has indicated the possibility of further collecting in Greece and Yugoslavia and the Germplasm Institute of Bari intends to collect further

in Italy and Greece. RCA, Tapioszele, will continue its programme of forage collecting in Hungary. WPBS, UK, intends to collect Lolium perenne and Trifolium repens in eastern countries of Europe. Although no further collecting is planned in Ireland in the immediate future, further collecting may be undertaken in specific areas when the current collection is evaluated. The meeting also noted with satisfaction that recommendations for collecting issued at the meeting in Carlow were near completion.

21. The following priorities for further areas and species were identified for collecting: i) high altitude areas within the Mediterranean zone, particularly on the islands of Crete, Corsica, Cyprus and Sardinia, ii) all forage legumes, Dactylis, Festuca and Lolium throughout Yugoslavia and Albania, iii) southeast Iberia for subclover, medics and Ornythopus, and iv) perennial Lathyrus in central and northeast Europe. In addition, the meeting expressed general interest in all forages in the USSR.

Collaboration of the European forage network with other regions of the world

22. Members reiterated that the process of individuals making contact with one another should be encouraged. The meeting considered that achievements of the Group to date had been significant and agreed that the time had come for the establishment of formal contacts with all principal institutes dealing with temperate forages in other parts of the world. The Group envisaged the first step as the exchange of databases. Discussions centred on offering the Group's expertise to Third World countries with interest in temperate forage species. Members recommended that ECP/GR/IBPGR identify ways of achieving these objectives.

Coordination of the European forage network after the end of Phase III

23. The Working Group agreed that the network of communication initiated through the activities of the European Forage Data Bases to deal with species/genera had now reached some self sustainability. The Group expressed its strong concern for the future of the European forage genetic resources network, if it proved impossible for it to meet regularly.

It emphasized that regular meetings provide unique opportunities to review progress, facilitate rapid exchange of information, identify common problems and find the best solutions.

The Group unanimously agreed that regeneration, core collections and in situ conservation had emerged as new aspects for further work in genetic resources and had been highlighted by the achievements of the European Forage Data Bases. These topics could not be dealt with efficiently at a national level only and therefore required continuing coordination by an international organization.

Other matters

24. A vote of thanks was given to IBPGR for its continuing support.

The Group wished to express its thanks to the forage group at INRA, Montpellier, for its warm hospitality and the excellent organization of the meeting.

APPENDIX I

LIST OF PARTICIPANTS

Chairman

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APPENDIX II

AGENDA

1. Opening Addresses
2. Election of Chairman
3. Adoption of Agenda
4. Review of activities since the working session held in INRA, Lusignan, 24 September 1987
   - 4.1 Presentation of the report and recommendations of the working session
   - 4.2 Achievements of European Forage Data Bases
     - 4.2.1 Registration of passport data
     - 4.2.2 Publication and distribution of European forage lists (in printed and computerized form)
     - 4.2.3 Mapping of collected accessions
   - 4.3 Progress in collecting
   - 4.4 List of standard varieties
     - 4.4.1 Reference varieties for forages genetic resources in northern and middle Europe
       - 4.4.1.1 Adoption and use of these varieties
       - 4.4.1.2 Distribution of these standard varieties through forage databases
     - 4.4.2 Standard varieties for forage legumes in the Mediterranean zone
   - 4.5 IBPGR progress on survey of forage genetic resources in the Mediterranean area
   - 4.6 An international network on medics

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APPENDIX II (continued)

5. Strategies for maintenance of forage genetic resources (Chairman: R. Reid)
   - 5.1 Introduction papers:

     Techniques of regeneration/multiplication in the field (V. Connolly et al.)

     Some observations on regeneration techniques using isolation compartments (B. Tyler)

     Pooling accessions: advantages and disadvantages (P. Guy et al.)

     In situ conservation (P. Weibull)

     Contributions of forage databases to maintenance of forage genetic resources: a documentation officer's point of view (J. Serwinski)

     Accurate identification of wild forage species in forage collections (N. Maxted and F. Bisby)

     Pre-breeding in genetic resources of perennial ryegrass (Lolium perenne L.) (C. Paul)
   - 5.2 Discussion and recommendations
6. Formulation of a workplan
   - 6.1 European Forage Data Bases
     - 6.1.1 Further registration and distribution of data
     - 6.1.2 Selection of a core collection
     - 6.1.3 Services of databases to breeders/researchers
   - 6.2 Safety duplication
   - 6.3 Recommendations for further collecting
   - 6.4 Use of standard varieties and exchange of characterization/ evaluation data.
   - 6.5 Collaboration of the European network with other regions of the world
   - 6.6 Recommendations on coordination of the European forage network after the end of Phase III
7. Other matters
8. Writing of report
9. Consideration of report and approval by the Working Group

APPENDIX III

EUROPEAN FORAGE DATA BASES

Bromus spp.	Research Centre for Agrobotany, Institute for Plant Production and Qualification, H-2766 Tápiószele, Hungary
Dactylis spp. and Festuca spp.	Plant Breeding and Acclimatization Institute, Radzikow, 05-870 Blonie, Poland
Trifolium subterraneum, and Medicago (annual species)	Servicio de Investigación Agraria, Apartado 22 06080 Badajoz, Spain
Lolium multiflorum, L. perenne and Trifolium repens	Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, UK
Lolium (annual species), Phalaris spp. and Vicia spp.	Laboratorio del Germoplasma, Consiglio Nazionale delle Ricerche, Via G. Amendola 165/A, 70126 Bari, Italy
Medicago (perennial species)	GEVES-INRA, La Minière, 78280 Guyancourt, France
Poa spp.	Institut für Pflanzenbau und Pflanzenzuchtung der Bundesforschungsanstalt für Landwirtschaft, Braunschweig-Volkenrode, Bundesallee 50, 3300 Braunschweig, FRG
Phleum	Nordic Gene Bank, P.O. Box 41, 230 53 Alnarp, Sweden
Trifolium pratense	Federal Agricultural Research Station of Changins, Route de Duillier, 1260 Nyon, Switzerland
Lathyrus latifolius, L. heterophyllos, L. silvestris and L. tuberosus	Institut de Biocénotique Expérimentale des Agrosystèmes, Av. de l'Université, 64000 Pau, France
Other Lathyrus spp.	Biology Department, University of Southampton, Southampton SO9 5NH, UK, and Institute de Biocénotique Expérimentale des Agrosystèmes, Av. de l'Université, 64000 Pau, France

APPENDIX IV

ADVISED FORMAT FOR RECORDING GENETIC RESOURCES DATA FOR EXCHANGE

Recommendations for the recording of genetic resources data for exchanging information at the international level were issued at an ECP/GR Workshop on Exchange of Information (Radzikow, Poland, October 1984) (Ref: AGP:IBPGR/84/157).

The following document recalls the general rules for recording of data with some small changes stemming from the last four years' experience on the exchange of genetic resources data.

At the end of 1980s the most popular medium for exchange of computerized data is a floppy disk compatible with the XT type of IBM series computers. A floppy disk of 5.25 inches with a capacity of 360 kbytes (double sided, double density) is recommended for such exchange. Exchanged files should be written into the disk using the MS-DOS COPY program (not backups).

The data set to be exchanged between genebanks should be recorded on a communication medium like a floppy disk as two files, the first one serving as a dictionary to the second, which contains the germplasm data. The proposed standard for formatting these two files is given below.

I. Dictionary file

```
                                    Bytes:
Records:
                1111111111222222--44444444455555555566666666
      12345678901234567890123 45--12345678901234567890123 4678

1   Descr. name of data file    YYMMDDName of genebank
2         N     S
3   Name of the 1st descriptor  n1  DN1  DS1            IDS1
.   .....                       .   ..   ..             ..
N   Name of the Nth descriptor  nN  DNN  DSN            IDSN


Record 1:

Byte:    1-40  Descriptive name of the data file
        41-46  Date of extracting of the data from database of delivering
                    genebank. Date should be recorded in format YYMMDD,
                    where
                    YY   = year, last two digits
                    MM   = month, expressed numerically
                    DD   = day, expressed numerically
        47-72  Name of genebank delivering the data
```

Record 2:

Byte:		
	1- 6	Number of descriptors (N)
	11	'Space' indicates fixed format, i.e. each descriptor has allocated fixed-length field in the record
	'_'	Underscore (or other separator) indicates free format, i.e. fields are not allocated to descriptors; instead, the descriptor states are recorded in as many characters as needed and they are separated by a 'non-space' character. It is recommended that '_' (underscore) character be used as a separator

Starting with the third record, the definitions of descriptors are recorded. Each descriptor definition is stored in a sequence of records, the first of which has the following fields:

Byte:		
	1 - 40	Name of descriptor. Name must begin with 'non-space' character
	41 - 44	Maximum length of descriptor ni
	45 - 49	Descriptor Number as used by delivering genebank (optional) DNi
	50 - 64	Descriptor Symbol as used by delivering genebank (optional) DSi
	65 - 80	International Descriptor Symbol IDSi (optional). This field refers to the internationaly agreed-upon descriptor lists. When the particular descriptor is on the international list the reference to it can be recorded here.

The remaining records of descriptor definitions are intended to provide a clear, unambiguous description of a descriptor and its possible states in a plain text. The format for these records is the following:

Byte:		
	1	'space'
	2 - 80	text

All records of the dictionary file are 80 bytes long.

II. Data file

The structure of this file is described by the dictionary file. A summary of the file attributes and format is given below.

1. The number of records is equal to the number of accessions.

2. A single record contains all the descriptor states for one accession.

3. The descriptor states are recorded in the order defined in the dictionary file.

4. Records are either fixed or variable in length depending on the format given in dictionary file record 2 byte 11.

5. For the fixed-length records, the position of each descriptor in the record can be calculated using the information contained in the dictionary file.

6. In the case of variable length records, the record must be searched sequentially to find the position of a particular descriptor. All descriptors are separated by separator given in dictionary file record 2 byte 11.

7. When the descriptor state is missing, the field allocated for the descriptor is left blank in fixed-length records; for variable-length records only the separator is used.

At the end of each record in both files, characters carriage return (CR) and new line (NL) must be included.

All information in both files has to be recorded as alphanumeric character strings.

An example of recording genetic resources data in files is shown below.

Dictionary file

```
                Bytes within the record:
                1111111111222222222-44444444455555555556666
      12345678901234567890123456789-12345678901234567890123456789012

  SECALE                              840710IHAR - RADZIKOW <CR,LF>
  10        -                                               <CR,LF>
  ACCESSION NUMBER                    6   1     NUM         <CR,LF>
   According to IBPGR definition, descriptor number 1.1     <CR,LF>
  ACCESSION NAME                      40  2     NAZ         <CR,LF>
   A name or other designation applied to the accession     <CR,LF>
  SPECIES                             15  3     GAT         <CR,LF>
  DATE OBTAINED                       8   4     DAT         <CR,LF>
   Date the sample entered the genebank, form DD/MM/YY      <CR,LF>
  ORIGIN COUNTRY                      3   5     KRP         <CR,LF>
   According to the FAO/IBPGR country code                  <CR,LF>
  PLANT HEIGHT IN CM                  3   6     WYS         <CR,LF>
  LODDGING RESISTANCE                 1   7     WYL         <CR,LF>
   Resistance to lodging recorded on a 1 - 9 scale          <CR,LF>
  SEEDS MASS PER PLANT IN G           3   8     MNS         <CR,LF>
   Average of 10 plants                                     <CR,LF>
  1000 SEEDS MASS IN G                3   9     MTZ         <CR,LF>
  PROTEIN CONTENT                     2   10    BIA         <CR,LF>
  Measured as percentage dry weight                         <CR,LF>

<EOF>
```

Data file: (Variable record length)

```
030954_HORTON_CEREALE_10/05/72_CDN_105_7_80_50_13_<CR,LF>
031125_DANKOWSKIE ZLOTE_CEREALE_05/05/75_POL_100_8_85_57_15_<CR,LF>
030839_KUNGS II_CEREALE_06/07/80_SWE_95_5_83_48_12_<CR,LF>
.
<EOF>
```

Data file: (Fixed record length)

```
030954HORTON                                CEREALE
10/05/72CDN105780 50 13<CR,LF>
031125DANKOWSKIE ZLOTE                      CEREALE
05/05/75POL100885 57 15<CR,LF>
030839KUNGS II                              CEREALE
06/07/80SWE95 583 48 12<CR,LF>
.
<EOF>
```


APPENDIX V

INTRODUCTION PAPERS ON STRATEGIES FOR MAINTENANCE OF FORAGE GENETIC RESOURCES

TECHNIQUES OF REGENERATION/MULTIPLICATION IN THE FIELD

by V. Connolly, J.G. Crowley, M. do Valle Ribeiro, TEAGASC, Oak Park Research Centre, Carlow, Ireland

The following contribution summarizes the procedures used and experience gained during propagation of forage grass (mainly Lolium) and legume (Trifolium repens) collections.

In the period 1979-83 an intensive programme of sampling old pasture ecosystems was undertaken in Ireland. This work was funded in part by the EEC. Table 1 lists the species and number of populations sampled. Two types of collection were made: (a) vegetative tillers and (b) seed. L. perenne and T. repens formed the major part of the collection, and in addition some populations of other species were also collected, as shown in Table 1.

Number of genotypes per population

The target number of plants per population was 100 for the vegetative collection and 80-100 seed heads per population for the seed collection. It was estimated that this number would ensure adequate sampling of the genepool and also provide sufficient seed for storage, field evaluation in replicated plots and for distribution.

In practice, the number of genotypes per population varied from 50 to 150 (Lolium) and 40 to 120 (Trifolium repens). The vegetative plant collection was prepared for propagation shortly after collection while the seed material was dried and stored for propagation at a later date.

All of the species involved in the collection are cross-pollinating and have efficient out-crossing breeding systems. Different methods of isolation were employed for the wind-pollinated grasses compared with that for T. repens (insect pollinated).

TABLE 1. Species and number of collections in the period 1979-83

Species	Material collected		
	Vegetative tillers	Seed	Total
Lolium perenne	382	147	529
L. multiflorium	3	2	5
Dactylis glomerata	22	31	53
Phleum pratense	10	14	24
Festuca pratensis	--	8	8
Festuca arundinacea	--	4	4
Festuca rubra	--	7	7
Poa pratensis	--	1	1
Trifolium repens	309	76	385
No. of sites sampled	392	159	551

1. Grasses

Isolation

Because of the large number of populations and the relatively large number of genotypes per population, it was decided to use isolation by distance for these wind-pollinated species.

Griffiths (1950) investigated the contamination due to wind-blown pollen on seed crops of Lolium. Contamination was very low after 25-30 m even in the absence of a pollen "barrier" crop. Poulsen and Pedersen (1982) found that winter rye is a good barrier crop and small plots of grass populations planted 10 m apart in the rye crop were adequately isolated. In our propagation system we used winter oats (variety Peniarth, long straw) as a pollen barrier crop and the populations of grasses were planted 30 m apart in both directions, i.e. 10 populations per ha of winter oats. Other crops such as winter rye or triticale would be equally good for isolation purposes. There are no exact guidelines to follow regarding isolation distance when using a pollen barrier crop because much depends on the crop itself -- length of straw, maximum foliage density (which should coincide with the pollination of the grass crop), wind speed and direction, etc. In one year we tested the effectiveness of this isolation by using male sterile ryegrass genotypes as test plants. Only two seeds were recovered from these plants, which indicates that the isolation was very effective.

Genotypes of each population were space planted in rows 500 mm apart between and within rows. Prior to planting out in the field, the individual genotypes were established and maintained in 100 mm pots. Field planting was normally done in October/November. In one year planting was delayed until February. This was satisfactory but overall seed yield was reduced (see Table 2).

Nitrogen

50 kg/ha at planting followed by 100 kg/ha in late February/early March. This is spread by hand because of the small area of each isolation. We have also used the small hand-operated "Hege" fertilizer spreader for this purpose. The level of nitrogen for the oat crop must be carefully regulated to ensure a vigorous crop that will not lodge. The amount used depends mainly on the previous cropping history of the area involved.

Weed control

The site chosen for propagation was free from grass weed species such as Agropyron, Agrostis, etc. This is essential. Herbicides used to control broad-leaved weeds in the oat crop were also used on the isolation areas. In addition, "Nortron" (ethofumesate) was applied to the isolation areas in February.

Disease control

Overall spraying of the oat crop for control of mildew and rhynchosporium and other fungal diseases - at least two applications per year. Additional spraying was used depending on disease levels. Where grass genotypes are space planted, infection by ergot (claviceps) can be serious, especially in Lolium. Separation of the ergot (sclerotia) from the seed is difficult. A regular spraying routine for the control of ergot is essential, beginning before anthesis. We have used "Mistral" (fenpropimorph) and "Sportak" (prochloraz) at two-week intervals commencing prior to anthesis and continuing throughout the flowering period.

Data collection

Date of flowering was recorded for the central seven rows of plants, i.e. 70 genotypes. In addition, visual assessment of spring growth was made in most populations. Because of the degree of disease control used during growth of the spaced plants no assessment of disease susceptibility was made.

Seed harvest

Harvesting of seed commenced when seed moisture content was 30-40%. Early harvesting of seed was desirable in order to avoid shedding. Because of the high moisture content the seed had to be conditioned with cold air and later with warm air to bring moisture levels down to 6-8%. The objective initially was to harvest, thresh and clean the seed from each genotype separately. The seed sample for storage would then be obtained by mixing equal quantities of seed from each genotype. This was done for some isolations at the beginning of the harvest period. However, the time and labour requirement for this procedure is so great it was not practical to proceed in this way. The remaining isolations were harvested in bulk. In difficult weather conditions individual plant harvest is almost impossible.

Seed yields

Mean seed yield in grams per plant is shown in Table 2. The yield per plant in 1982 was much lower than in 1983. This was due primarily to the timing of collection and subsequent transplanting of vegetative material in the field. In 1981 the tiller collection was made in September. The plants were prepared and transferred to isolation areas in February of the following year. Tillering was poor and seed production relatively low in 1982. The second phase of collection of vegetative material was in March/April 1982 and plants prepared and transplanted in October. These were much more vigorous plants with many tillers and consequently much better seed yields were obtained in 1983.

TABLE 2. **Seed yields (grams/plant)**

	Lolium perenne		Trifolium repens
	1982	1983	All years
Mean	5.1	15.0	3.4
Range	1.0-16.0	8.0-26.0	0.78-8.8

2. Legumes - T. repens

This species is pollinated by bees. Some studies suggest that bees are less effective in distributing pollen than wind. However, all of these results are derived from studies of seed propagation on a commercial scale where blossom density is high and adequate to meet the needs of the beehive. in such circumstances bees forage over a restricted area. Where small numbers of spaced plants are involved with low blossom density then spatial isolation would require separation of population by at least 200 m to reduce the risk of serious contamination (Johansen, 1968). For this reason, it was decided to use bee-proof pollination chambers for propagation of this species.

Isolation houses

These houses were based on a polythene tunnel structure 7.5 m x 5.2 m x 2.4 m (length x width x height at apex). Some were covered with bee-proof "tygan" nylon mesh, in other types the structure was covered with plastic and the ends sealed with tygan mesh. Because our summer temperatures are frequently low we have found that the pollination houses covered with plastic are better than those where nylon mesh is used throughout. This is largely due to higher temperatures, better bee activity and more control over seed ripening and harvesting during wet weather.

Selection between populations

Because the number of collected ecotypes of T. repens was much greater than the number of pollination houses (20) available, it was necessary to do some prior selection. The selected populations were then regenerated over a number of years. All the collected ecotypes were space planted in the field at 1 m x 1 m centres for observation. Leaf size, date of flowering, blossom density, petiole length and stolon thickness were estimated. Although there was some variation in leaf size, all populations belonged to the small-leaved type with fine, dense stolon growth pattern. There was much variability within and between populations for other characters such as blossom density and seed yield. The objective was to select populations that maximized genetic differences between populations (Tyler, 1982). Passport data obtained during collection was combined with field assessment of spaced plants in order to select the populations for propagation.

Preparation

Stolons from each genotype of the selected population were transferred to 250 mm pots using sterilized soil/sand/peat-moss compost. These pots were then isolated in the pollination houses. If seed harvest from each plant is planned then the maximum number of genotypes per house is approximately 100. Greater numbers can be accommodated but this leads to difficulties at harvest time. Automatic control of water to each pot was maintained using a drip system.

Insect control

Aphids can be a major problem in these pollination houses, especially those covered with polythene. It is essential that plants are free of infection prior to the introduction of bees. "Bladafum" (sulfotep), smoke generators (for polythene houses) plus "Rogor" (dimethoate) was effective before bees were introduced. Control of aphids during flowering was done with "Aphox" (pirimicarb) applied in the evening.

Pollination

Nucleus beehives containing approximately 2 500-3 000 bees were introduced to each pollination house shortly after all genotypes had flowered. This delay in introduction of the nucleus hive until all genotypes had commenced flowering will reduce or eliminate the risk of genetic shift that might occur due to assortative mating if there is a broad range of flowering times within the population. In general, pollination and seed set was good.

Harvesting

Each genotype was harvested separately and equal numbers of heads combined to give the final seed sample for storage and evaluation.

Seed yields

Average yield of seed per plant over all populations and years = 3.4 g. Range = 0.78 - 8.8 g (Table 2). The wide range of seed yields is in part due to genetic differences between and within populations but major environmental differences can also arise between pollination houses, especially where disease/insect control is not adequate. This can severely depress seed yield.

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SUMMARY: REGENERATION OF CROSS-POLLINATING SPECIES

A. Grasses

1. Preparation: 100 (approximately) genotypes planted in 100 m pots. All material to be prepared in as short a period of time as possible. Include two control varieties as standards.

2. Isolation: Spatial isolation, populations 30 m apart and surrounded by a pollen "barrier" crop. Winter oats, rye or triticale.

3. Site: Essential that site is free from perennial weeds.

4. Planting: September/October. All material to be treated uniformly from time of preparation. Planting density 500 x 500 mm.

5. Fertilizer: High nitrogen required on isolation area to ensure good, vigorous plants with good seed production potential. N_2 at sowing and end February/early March. Nitrogen level on pollen barrier crop must be sufficient to ensure a vigorous crop that will not lodge.

6. Herbicides: Standard cereal herbicide to control broad-leaved weeds plus "Nortron" (ethofumesate) on isolation area in February.

7. Fungicides: Control of mildew and rhynchosporium in both the isolations and the cereal barrier crop (standard fungicides). Essential also to prevent ergot (claviceps) infection in grasses. "Sportak" (prochloraz) or "Mistral" (fenpropimorph) at two-week intervals prior to anthesis and throughout the flowering period.

8. Harvesting: Individual plant harvest desirable but may not be possible because of labour requirements. It is feasible only when number of populations is small.

B. Legumes

1. Isolation: Spatial isolation not practical. Special bee-proof pollination houses used. If number of populations is large, it may be necessary to do prior selection to reduce the number for regeneration.

2. Preparation: Stolons of single genotypes rooted in 250 mm pots using sterilized compost. Approximately 80-100 genotypes per population (propagate in November/December).

3. Aphid control: Prior to introduction of bees - "Rogor" (dimethoate), "Desis" (deltamethrin) or smoke generators (sulfotep) in polythene-covered houses (with tygan ends temporarily sealed). "Aphox" (pirimicarb) during pollination period.

4. Fungicides: "Sportak" (prochloraz) and "Benlate" (benomyl) to control mildew if this is a problem.

5. Pollination: Nucleus beehives, 2 500-3 000 bees per hive introduced when all genotypes have flowered.

6. Harvesting: Individual plant harvesting is possible especially in the polythene-covered houses, since harvesting is independent of weather conditions.

APPENDIX V (continued)

SOME OBSERVATIONS ON REGENERATION TECHNIQUES FOR FORAGE GRASSES USING ISOLATION COMPARTMENTS by B.F. Tyler, Welsh Plant Breeding Station, UK

The use of isolation compartments is one technique possible for the initial multiplication and regeneration of wind-pollinated genetic resources, including grasses. At WPBS, 96 of the smaller type (floor area 1.4 m x 1.2 m) are available for regeneration, the facility being shared with the plant breeders. Practical aspects of the regeneration scheme in use in 1981 are reported in the proceedings of a EUCARPIA seminar given in Denmark in that year (Tyler, 1982). At that meeting another paper was given that considered the more theoretical aspects of the scheme (Breese and Tyler, 1982). The purpose of this paper is to indicate developments that we have adopted since that report.

Our primary objective then was to obtain the maximum seed yield from an island with the minimum genetic change in agronomic characters. Since then, however, some concern has been expressed that loss of low frequency alleles might occur using this method. This concern was occasioned by the rapid expansion of biotechnology and the fear that our resources may be depleted of alleles that might be valuable in the future. Although our major concern is still the production of sufficient seed for evaluation of potential breeding material we decided to investigate certain procedures in use to see if they significantly contributed to genetic erosion and, if practically possible, to modify our scheme.

The main precautions taken to avoid excessive genetic change in regeneration are:

1. Avoid contamination by foreign pollen.

2. Avoid random drift.

3. Secure random mating.

4. Avoid shift through natural selection.

5. Ensure maximum yields of good quality seeds and high standards of seed storage to reduce number of subsequent generations required and hence reduce risks from 1-4 above.

These points were considered in some detail by Breese and Tyler (1982) and some of the issues raised have been investigated in light of the recent concern.

In the opinion of Gale and Lawrence (1984), the only way to ensure the retention of maximum genetic diversity is to use a controlled mating system, such as bi-parental mating. There are considerable practical difficulties in adopting this procedure in forages, due to the wide variation in flowering time within populations and hence the impossibility of ensuring random mating and the consequent resultant low seed yields, but more important is the considerable increase in time and labour this would involve. For practical reasons this method was rejected.

Thus, having decided to retain the uncontrolled polycross technique, we looked at one aspect of this that was likely to have the most profound effect on genetic stability, i.e. differential reproductive capacity of the genotypes and its effect on paternal and maternal contribution in the subsequent generation and on random mating.

Paternal contribution

We made observations on a small island consisting of 14 genotypes of Festuca pratensis collected in September 1986 from Austria and multiplied for seed in 1987. Daily visits were made and the date of anthesis and spread of antheses on each inflorescence were recorded.

The conclusions were:

1. The spread of inflorescence production within a genotype and the range of anthesis within an inflorescence were sufficiently wide that only three genotypic combinations out of 180 were not theoretically possible.

This relatively large spread of both inflorescence production and anthesis would appear to favour random mating.

2. The most striking observation was the high reproductive activity of one plant, genotype 3, where a total of 17 inflorescences were produced over 14 days, most in the first three days, compared with an average of four inflorescences for other genotypes.

This imbalance would appear to restrict the genetic composition of the pollen available for pollination and thus militate against randomness. For example, for the first six days 50% of the pollen would be derived from genotype 3, during which period one-third of the inflorescences of the other genotypes were receptive for pollination. This is likely to result in genetic imbalance in paternal germplasm with genotype 3 being favoured at the expense of the other genotypes. The bias due to genotypes with abnormally high reproductive capacity could be negated, to some extent, by reducing the number of inflorescences to the average of the population, before anthesis.

As a result of this and other circumstantial evidence on the effect of paternal and potential maternal contribution, an experiment was set up in 1987 in an attempt to detect and measure the extent of any change.

Maternal contribution

A collection of 90 plants of an extreme pasture-type perennial ryegrass was made. Thirty plants were allocated at random to three separate polycross islands and seed harvested from each plant in 1988. The weight of seed derived from each plant was recorded. By bulking all of the seed from each polycross, the normal practice to obtain sufficient seed for agronomic evaluation, seed distribution and storage, the dominance of a few genotypes with high reproductive capacity can been seen. In each of the three replicate islands the five highest-yielding genotypes contributed approximately 50% of the seed, i.e. in a bulk sample every second seed would have been derived from one of the five plants. In addition, 90% of the seed was derived from approximately 50% of the population, reducing considerably the chance of the other half appearing in subsequent generations. In a well-balanced polycross there should be an equal chance of selecting from any one of the mother plants. In this example this is obviously not the case.

The experiment is planned to compare populations derived from F_1 seed lots derived in different ways using morphometric and isoenzyme techniques. We will be looking at a number of characters, but in particular any effect on the major agronomic and physiological characters.

Derivation of F_1 seed

1. Unbalanced bulk - the bulk comprising all seed produced by the polycross irrespective of the contribution from each mother plant.

2. Balanced bulk - a bulk consisting of equal amounts from each mother plant.

3. Individual seed - a polycross derived from the seed of each mother plant.

F_2 seed will be produced from these different bulks and compared. Initial results are not anticipated until the end of 1989 or 1990. In the interim we have modified our regeneration procedure on our most valuable accessions. All the usual precautions against shift, drift and contamination are taken but seed is collected from each mother plant, a small amount (± 0.5 g) retained in long-term storage for subsequent regeneration and for the construction of a balanced bulk (if required), the remainder being bulked for evaluation and distribution.

If the experiment shows no significant change in the major characters the use of an unbalanced bulk would be perfectly acceptable for our purpose, but if this is not the case, we will have to reconsider our technique. Regeneration with minimal genetic decay can be obtained from the individual mother plants, as can small, balanced samples for research.

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APPENDIX V (continued)

POOLING ACCESSIONS: ADVANTAGES AND DISADVANTAGES

by Pierre Guy*, Marc Ghesquière*, Gilles Charmet**, Jean-Marie Prosperi***

Introduction

Is a pool for genetic resources a means of keeping genetic variability at lower cost?

This issue may be discussed with passion, all the more so since the experimental data are lacking and the situation may vary from one species to another. Our reflections are linked with two constraints:

- the number of accessions that we may define, maintain, and dispatch is limited. In this study, we assume that the manager of a genebank works on a constant number of accessions, and

- maximum variability must be maintained.

As soon as the number of accessions exceeds the processing capacity of the genebank a choice has to be made, i.e. either to eliminate extra accessions or form a pool. We do not have enough knowledge to compare objectively these two courses. We will therefore only try to define some conditions where it is possible and/or useful to form a pool.

1. Elements of selection

It is very difficult to preserve all samples collected for accessions present in a genebank. Among the steps involved in one genetic resources operation (collection, multiplication, conservation and storage), the most limiting factor is always regeneration. In these circumstances, pooling of different accessions may be of interest, because it should increase i) the number of plants that are multiplied, ii) genetic variability, and iii) reduce the number of multiplications.

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Pooling method mainly concerns multiplication for distribution. Those in charge of genetic resources must keep, as long as possible, one unregenerated subsample of each original accession in the best possible conditions.

Different factors can influence the decision of whether or not to pool. These are species biology (such as breeding system) and the purpose of storing the genetic resources.in question.

A. Specific constraints

The main constraint is reproduction of the species. In fact, it is quite easy to multiply, using small areas, a large number of accessions of very autogamous species, like medics. A few precautions must be taken to avoid mixing at harvesting, or multiplying the same species side by side.

On the other hand, the need for isolation of allogamous species restricts the number of accessions that can be multiplied in the same areas. Under these conditions, control of pollination is more difficult. For these species the effective size of population must be larger to limit problems of consanguinity. But the difficulty depends on the species: maize, for instance, because it is monoecious, is very easy to cross-pollinate and allows good control of successive generations. An alternative method between pooling and multiplying small, isolated populations is the multiplication of small populations with sister-brother crossings in order to preserve original accessions. Excess seeds can then be mixed for distribution.

This work is more difficult for Gramineae with anemophilous pollination like Dactylis or Festuca. But it is possible with isolating cages or by multiplying in cereal fields.

For insect-pollinated species, like alfalfa, multiplication of a great number of populations with few plants, under strict control of pollination, is quite impossible. Control of insects is difficult in small isolation cages and alfalfa has a high level of autogamy (between 10 and 60% depending on the environment and the origin of the ecotypes). All these constraints make the employment of micro-multiplication techniques difficult.

It is evident that if the species is very rare and the number of accessions restricted, the only solution is to multiply all available accessions under isolated conditions with great prudence.

B. Uses of genetic resources

The main uses of genetic resources are for plant-breeding and conservation of important but often unknown characteristics for the future. In the former case, all methods of classifying genetic resources, preserving them and making them more accessible for plant breeders are worth investigating. Pooling is one possibility here. Pooling different accessions taking the precautions mentioned below can be compared with the first step of recurrent selection. But this work must take into account the ideotypes of plant breeders. For example, for prostrate Medicago sativa (alfalfa), constitution of different pools with different geographic origins will be an important tool for plant breeders. It is the same for Gramineae with different groups of precocity or for maize with the populations sources work carried out in France by INRA and private institutes.

TABLE 1. **Different types of pool**

Reason for pool/pool type	Explanation
Choice	For important agronomical characteristics: e.g. prostrate lucerne for grazing
A priori	To preserve variability for the future e.g. low-yielding plants (turf!), frost-susceptible plants
"Natural"	Pooling of duplicates or quasi-duplicates e.g. landraces of lucerne (A. Birouk, Hassan II University, Morocco) e.g. wild populations of perennial ryegrass (G. Charmet, INRA, France)

But the second aim of preserving genetic resources will be reached only if we preserve and also multiply all the accessions that do not correspond to these ideotypes, e.g. frost-susceptible plants for Mediterranean or tropical countries or very low-yielding plants that are of no interest as forages but are very valuable as turf grasses.

One way to make good and interesting material is to pool different accessions on the basis of geographic, morphological and, if available, electrophoresis characteristics, with the aim of preserving maximum variability for the future. But one must keep in mind that any regeneration results in some deviation from the original material, whatever method is used, either through genetic drift, uncontrolled selection or modification of the population structure.

The pooling method is strongly conditioned by the amount and type of information about accessions. It is possible to multiply different accessions under isolated conditions with relatively poor information except for that regarding floral biology. But it is totally impossible to pool without detailed information on everything from site data to agronomic characteristics.

2. Information to be taken into account

A. Levels of information

It is possible to obtain several types of information on material. These are listed here, from the easiest to obtain to the most difficult and costly:

1. Geographic information: known as "passport data": location, altitude, etc. Ecological data: including natural (soil, climate) and artificial (past history, management, etc.)

2. Phenotypic evaluation: two kinds of traits can be measured in what are called "populations":

   - Morphological traits, which are generally quite heritable and could lead to a more precise taxonomy at the intra-specific level.

   - Traits of agronomic interest like vigour, disease resistance and drought tolerance. Most of these are less heritable and high Genotype x Environment interactions are frequent. Reliable estimates of "population" values (through variance analysis) for such traits can thus only be obtained from replicated multi-environment trials or from measurements taken under standardized conditions. If this is not the case, the agronomic description only has value in the range of environments used for the evaluation.

     Agronomic characters may be used to obtain a "typology" of the material through multivariate methods of classification.

3. Genetic evaluation: here again two kinds of genetic method can be employed:

   - Genetic markers using qualitative, single-gene dependent traits like isozyme frequencies.

   - Quantitative genetics on polygenetic-controlled characters such as the agronomic ones described above.

Reliable estimates of variance-covariance components need a considerable amount of work and can thus only be planned for a limited number of populations.

B. Choice of classification criteria

First it must be pointed out that there are obligatory ways of partitioning such as by species, ploidy level, flowering time, etc. which condition the ability of one type to mate with another.

1. Geographic data is associated in our mind with the concept of "isolation by distance" (Wright, 1951): two populations can be considered as different if the distance between their site is large. But the available data on wild populations of wind-pollinated plants generally indicate that neighbourhood size varies from some 10 m^2 to about 1 000 m^2 (see Brunel and Rodolphe, 1985). Taking this neighbourhood parameter into account would lead to an infinity of distinct "populations" we cannot distinguish in practice. In "country populations" of half-domesticated species like red clover or lucerne, seed exchange by farmers could allow enough geneflow to homogenize a population over a larger area.

2. Ecological factors may often enhance genotypic differentiation even between adjacent populations through natural selection (Jain and Bradshaw, 1966). The question that arises with a lot of ecological data is: Which ones are pertinent, i.e. exercise an effective selection pressure? The answer requires comprehensive knowledge of the biology and physiology of the plant.

   The combination of geographic distance and some ecological factors may be helpful for a preliminary grouping before further studies, but it is rarely used for pooling without agronomic evaluation.

3. Multivariate clustering methods applied to morphologic and agronomic traits have been widely used to classify a high number of accessions (e.g. Veronesi and Falcinelli, 1988). When based on agronomic traits, this typology is dependent on the choice of, and the relative weights given to each, trait and therefore on the "ideotype" the plant breeder has in mind. Such a method, leading to clearly defined types, is of direct interest to breeders: instead of asking the genebank for all the populations bearing similar characteristics, the breeder has only to manage a few "pools" with the desired traits.

   A possible advantage of the pool over separate populations is maximized genetic variance, allowing some traits to be improved within a given type (e.g. prostrate lucerne for grazing, slow growing grasses for amenity use, etc.).

   Since these phenotypic traits can be used alone, they are very often taken together with the former geographic and ecological data; this can be done either in a quite subjective way or by introducing constraints in the clustering method (Charmet et al., 1987).

4. The information supplied by genetic markers can be used in two ways:

   - for a preliminary survey of the division of the overall population into subpopulations by genetic structure (Wright, 1965), estimation of geneflow, etc. The computation of F statistics may help us determine the adequate size of the pooling area.

   - to verify whether the multivariate typology reflects genetic relationships such as those indicated by Nei indices (Nei, 1972) or is the result of evolutionary convergence. In the former case, pooling may be considered as natural, just rebuilding what evolution has not yet broken down. In the latter case, pooling is more artificial and can be seen as the first step in a plant breeding process.

Although quite laborious, the routine use of genetic markers alongside agronomic evaluation would be the best way to obtain homogeneous and genetically related pools of populations.

A demonstrative example of the combined use of geographic, ecological, agronomic and isozyme data for the constitution of pools from Morocco lucerne populations is given by Birouk (1987) and Birouk and Guy (1986).

Since these pools are to be used by plant breeders, their genetic parameters for quantitative traits are of primary importance. But little is known about the behaviour of genetic parameters after pooling several populations. We can suggest that pooling populations showing no differences in their variance-covariance matrix (as tested by Hullback) may lead to a new population having the same parameters. But this approach is time-consuming and costly because of the high number of families to be studied.

If no genetic parameters are available for the initial populations, these are to be established directly from the pool, but care must be taken that the new population has reached a panmictic equilibrium.

Another way could be to verify whether populations that have similar traits have homogeneous genetic parameters or not. Experiments are therefore carried out to compare genetic variance and covariance before and after pooling using several types of information: agronomic-geographic, agro-ecological, agrogenetic distances, etc.

3. Some observations on gene maintenance in pools

Once several ecotypes have been chosen to be gathered and intercrossed to form a pool on the basis of the relevant above-mentioned criteria, it can be asked in what way and to what extent genetic variability will change.

Few studies report evolution of genetic variability over multiplication processes. Most of them deal with synthetic varieties, that is with a rather narrow genetic base, from theoretical expectations, and are more concerned about maintenance of expression of some selected characters, such as vigour, than about maintenance of an overall amount of genetic variability per se (Gallais, 1967 and 1970; Busbice, 1970). More recently, Hayward and Abdullah (1985) showed that improved traits of synthetic varieties may return very quickly to their original levels. They suggested that not only are the effects of genes involved (probably dominance and epistasis in this case) but also some negative correlations with genes related to fitness (soluble glucide content and number of seeds/plant). Conversely, use of very large genetic base populations for recurrent selection in maize cuts the difficulties of maintaining and breeding from such a large amount of genetic variability (Kaan, unpublished).

Given the goals of pool constitution, let us try to review briefly the main interacting factors involved in gene evolution and see which of them can be managed to preserve as much genetic diversity as possible in pools. This diversity may change according to two mechanisms well known to population geneticists: genetic drift and selection. They occur independently but when their effects are combined, they may lead to drastic allelic frequency changes, including allelic fixations and losses, and consequently bring about important modifications of the phenotypic expression.

A. Theory

1. Genetic drift

Most of the factors responsible for genetic drift have been identified: mating system, ploidy, allelic frequencies, sampling and panmixia, but often not all their parameters are accurately quantified and even if they were, it would be impossible to act on each of them:

- This is evident for the first two factors, unless laborious modifications of the plant biology are undertaken (auto-incompatibility, male sterility, polyploidization, apomixis, etc.) but predominant allogamy and polyploidy in most forage grasses can be regarded as advantages, aiding retention of many alleles.

- Most of the time, allelic frequencies or number of alleles by locus are undetermined because we do not know how, and for which locus, polymorphism is maintained in the ecotypes we have to multiply (random processes for selectively neutral alleles or selection for adaptative traits). However, regardless of the theory, we can be sure that at least some alleles are present in low frequency at each polymorphic locus. This is the main reason why, when maintaining all ecotypes individually is not possible, gathering them in some pools is a method, per se, of limiting the genetic drift of the material as a whole. But, as discussed above, the efficiency of this strategy is much enhanced when ecotype clustering is quite close to the true genetic structure. So rare alleles may be well protected from drift in suitable pools, due to high frequency recovery, whereas other alleles, spread widely among the pools, will certainly be sampled.

- In error sampling, the use of large and equal numbers is an obvious precaution taken to avoid serious discrepancies, although this is not always possible. Sampling should not be carried out regardless of mating system: in outbreeding species for instance, and provided panmixia is assumed, sampling of parental plants can be reduced when number of seeds per plant increases, but more than 5 seeds/plant gives very little gain in accuracy of the sample gene frequencies (Marshall and Brown, 1983).

- Finally, panmixia during multiplication processes is needed to prevent evolution of gene frequencies. A means to ensure it is to maintain the effective population number of the pool as constant as possible, or at least to minimize the effects of the main factors that could reduce this effective number. From theoretical projections given by Crow and Kimura (1970) and computer simulations, Bray (1983) illustrates different panmixia departure situations due either to sampling variance of the parents or to their gametic (male and female) contribution variance. It is clear that each of these components may combine and cumulate from one generation to the next, and thus act as so many irreversible bottlenecks of genetic variability.

2. Selection

Evolution due to selection in the pools is a result of many factors, as for genetic drift, but including gene effects and selection coefficients. As for many parameters occurring in gene drift, selection coefficients during multiplication are not known and are practically impossible to estimate. But, rather than make estimates, it is important to have some idea of the discrepancy between new selection coefficients and those that have occurred mainly on the collecting sites of the ecotypes. Provided this discrepancy is not so large as to cause serious elimination of living material, some passive mechanisms such as polyploidy, mating system, gametic disequilibrium, linkage and even level of residual genetic variance exist to prevent marked genetic evolution. In the case of adaptive traits, most genetic variance is probably additive and therefore restricted to a sufficiently low level to limit reversion of the traits. This again is why maintaining pools with suitable genetic bases, mainly constructed on adaptive traits and/or the ecological pattern of the collecting sites, would be quite a good strategy to minimize genetic evolution by selection.

However, keeping in mind that the genetic bases to be managed are not too large, pools should not lead to a redution in the effective number resulting in inbreeding effects that may add to genetic drift.

B. Practice

We have seen that factors affecting genetic variability in multiplication processes may be numerous. But most of them can only be considered from a theoretical point of view; taking them into account after preliminary studies would require a great deal of time and effort, even if it were possible to undertake such studies. Nevertheless, some practical recommendations may be made for the factors we can influence:

- As far as selection is concerned, no serious evolution should be expected if previous selection conditions are only relaxed. But much care has to be taken to prevent the occurrence of new directional and strong selection coefficients, which are not always obvious. One way of achieving this could be to split multiplication, in terms of both time and space, to minimize the effects of selection; but this is likely to be too expensive. A better method would be first to identify the main critical selective factor and prevent its action.

- Being very careful of panmixia conditions should not be too restricting a way of limiting drift effects, provided that each level of gene sampling is quite well controlled. In fact, we should keep all maternal progenies of the pool components separate, even if seed bulks would be more convenient for breeders.

- It is important, as already stressed, to have some idea about the genetic differentiation of the material because this can determine the suitability of group ecotypes and therefore influence the efficient preservation of genetic variability in the subsequent pools. But the problem remains to determine which relevant criterion or level of genetic variability to use in order to highlight this genetic structure.

- Two questions are relevant to any discussion about the need to maintain pool diversity: what is the status of the genetic resources of the species under consideration, and what are the potential uses of the pools and in what terms? It seems evident that panmixia survey in pool management would not be so great a problem in the case of unthreatened or already quite well collected species or those only used by breeders for some particular traits.

If one chooses a strategy of maintenance of genetic resources based on pool management, three important points, in our opinion, can be made:

- Gene maintenance has to be considered as a whole, taking into account genetic context of the species, level of genetic evaluation used and potential utilization by plant breeders.

- Practical management of pools must therefore be discussed in terms of optimization, i.e., every gene may not need to be retained.

- So, except for the theoretical recommendations to preserve as much genetic variability as possible, any technical maintenance has to be thought through and adapted, species by species. Nevertheless, many experiments are needed to deal more practically with maintenance method efficiency. Genetic markers, which electrophoresis now provides us with, could be extensively and profitably used to survey gene restructuring in pools and how this can be related to changes in some quantitative traits.

Conclusion

We consider that the main aim of maintaining genetic resources is always, over a more or less long-term period, varietal creation. The preservation of cultural patrimony (ethno-agronomics, etc.) is a worthwile objective but is not the primary concern of genetic resources maintenance. The pooling of genetic resources does not provide an easy technique. It requires good general knowledge of the species whatever our chosen strategy might be. It requires information on the genetic material to be grouped together. Without this knowledge, no pool.

Although we have tried to discuss aspects of the problem with which we are familiar, examining the concept of a pool of genetic resources has served to underline the gaps in our theoretical and experimental knowledge (the size of neighbourhood, the structure of variability, the effects of natural selection, the restructuring of the genome). By comparison with sampling, which means the elimination of accessions, the pool justifies itself if the hope of higher variability is greater than the risk of deterioration of the internal genetic balance and if the advantage of an increase in hidden genetic variability is greater than the danger of a decrease in expressed, accessible phenotypic variability.

The pool is more a constraint than a choice. For fodder plants, it should be accompanied by in situ preservation, as well as long-term storage of small samples of components.

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APPENDIX V (continued)

IN SITU CONSERVATION by P. Weibull, Weibullsholm Plant Breeding Institute, Landskrona, Sweden

Genetic resources can be conserved in genebanks (ex situ) or in nature (in situ). The pros and cons of in situ conservation of wild crop relatives have been discussed by Prescott-Allen and Prescott-Allen (1982) and their arguments are relevant for forage crops as well.

So far IBPGR has been concerned primarily with ex situ conservation and has left the task of in situ conservation to other international organizations, e.g. IUCN and WWF. The IBPGR policy statement on in situ conservation of wild crop relatives (1984) explains the IBPGR position.

The Nordic Gene Bank is presently about to set up an in situ conservation scheme for genetic resources in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden). Several forage crop species occur in a wild or semi-wild state in this area. An in situ working group will be set up with the task of making an inventory of existing nature reserves and other valuable areas, of their botanical composition, etc.

In view of the recent large decrease in the acreage of natural meadows and pastures in Sweden, the National Environmental Board started project "NOLA" in 1986 to conserve valuable areas.

Under this project tenants/owners of valuable areas sign a contract for a minimum of five years and receive economic compensation for the restoration and management (cutting and grazing but not fertilization) of valuable areas.

Presently, some 44 000 ha are conserved in this way at a cost of 18 million Swedish crowns. At the same time, a botanical inventory is being compiled.

Even if the NOLA project is not primarily aimed at conserving forage crop genetic resources, the Nordic Gene Bank can profit greatly from this habitat conservation scheme.

In situ conservation

Advantages:

- Continued co-evolution
- Possibility of studying the ecology of the species (tolerance to drought, salinity, parasites, etc.)
- Can be combined with other objectives (habitat protection)
- Successive collections possible
- Avoids space-consuming storage and time- and cost-consuming regeneration in the genebank

Disadvantages:

- May be difficult to conserve adequate genetic variation
- Vulnerability to human activities (development, population, growth)
- Access may be difficult for breeders
- Management costs may be high. Difficulties in carrying out proper management

This summary is based on the work of Prescott-Allen, 1982

IBPGR Policy Statement on In Situ Conservation of Wild Crop Relatives (1984)

In situ methods are preferred for wild species, which have germplasm that at the present time cannot be practically maintained in genebanks.

The Statement acknowledges the work by other international organizations, e.g. the International Union for Conservation of Nature and Natural Resources (IUCN).

IBPGR is to be concerned with and to support research on:

- Taxonomy of wild crop relatives
- Ecogeographical surveys as components of in situ programmes
- Conservation objectives for individual species and populations on a site-specific basis
- Assessment and monitoring of populations of wild crop relatives already conserved on an in situ basis

NGB responsibilities

The Nordic Gene Bank (NGB) has the task of preserving genetic resources of agricultural and horticultural crops in the Nordic countries (e.g. Denmark, Iceland, Norway, Finland and Sweden).

The NGB has just begun the planning of an in situ conservation scheme.

The indigenous genepool contains forage crop genera like:

Agrostis, Dactylis, Festuca, Phleum, Poa and Trifolium.

Reserves set aside for in situ conservation of certain populations will be designated base collections for accessions representing those populations.

The NGB will form an In Situ Working Group which will carry out the following tasks:

1. Inventory existing nature reserves and other valuable areas
2. Inventory plant species in these areas
3. Inventory meteorological and geological data
4. Produce documentation on intraspecific genetic diversity

These tasks are to be carried out in cooperation with official authorities and institutions as well as societies for the protection of flora and fauna (Dr. Stig Blixt, pers. comm.).

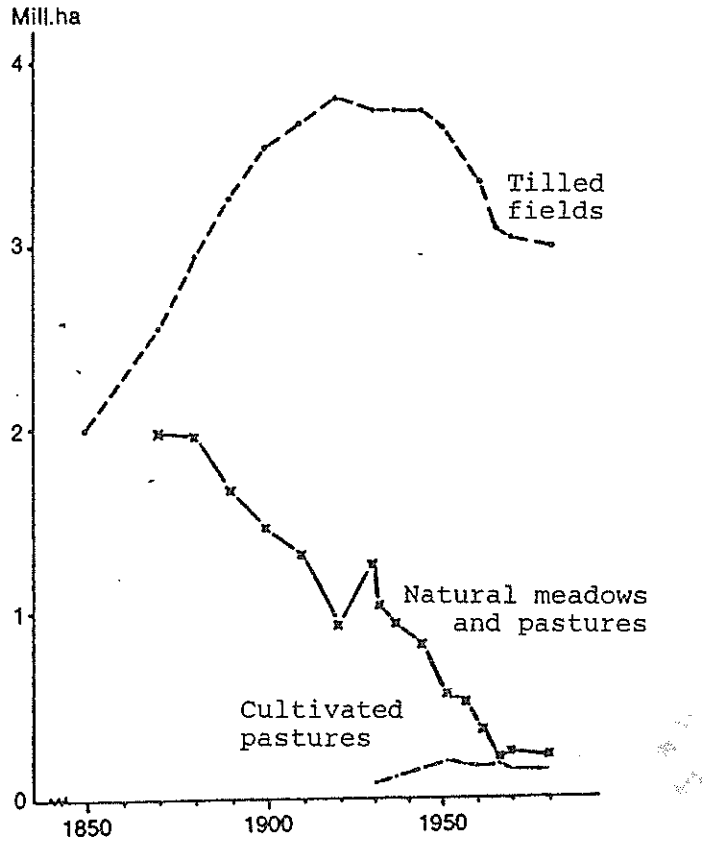

Figure 1. The area (in millions of ha) of tilled fields, natural meadows and pastures, and cultivated pastures in Sweden, 1850-1980

NOLA

Background

In recent years there has been a large decrease in the acreage of natural meadows and pastures in Sweden as a result of intensified farming and forestry.

Object

To conserve valuable natural meadows and pastures in Sweden.

Responsible authorities

- The National Environmental Protection Board
  (Administers total budget)

- The County Administrations (24 in all in Sweden)
  (Select areas)

Legal NOLA contracts between a county administration and the tenant/owner of an area have a minimum duration of five years, and are not applicable to nature reserves.

Economic grants are available for:

1. Restoration of areas where maintenance has been neglected (fencing, removal of trees and shrubs, water holes, etc.)

   Maximum: 50% of costs

2. Annual maintenance

   Cutting, maximum 600 sek/ha/year
   Grazing, maximum 300 sek/ha/year

3. Minimum: 1 000 sek/object

TABLE 1. Growth of the NOLA scheme, 1986-89

Year	Grants (million sek)	Total area (ha)
1986/87	4.0	11 000
1987/88	8.5	21 000
1988/89	18.0	44 000

Aim: 60 000 ha
1 sek = 9 p (GB), 15 c (US) (1989)

Main criteria for NOLA grants

A. Area must be well maintained, typical of its region, and have a long history of maintenance.

B. Area must be capable of being restored after intensified maintenance (cutting/grazing); a long history of maintenance is important.

C. Area must have endangered or rare habitats, vegetation types and species.

D. Area must have rich fauna and flora, dependent on maintenance.

E. Area must be of exceptional quality.

Areas should not be fertilized.

CONTRIBUTIONS OF FORAGE DATABASES TO MAINTENANCE OF FORAGE GENETIC RESOURCES: A DOCUMENTATION OFFICER'S POINT OF VIEW

by J. Serwinski, Plant Breeding & Acclimatization Institute, Radzikow, Poland

1. Overview of activities

During the first meeting of the Working Group on Forages in February 1984, in Greece, a Minimum Passport Data Sheet was defined. This sheet was used to facilitate the transfer of data between the European institutes. The institutes which were given the task of creating and maintaining databases for particular species started filling up computer files with data from such sheets. Only in a few cases was exchange of data between institutes performed in computerized form using magnetic tape or floppy disk.

Exchange of genetic resources data rapidly increased after the working session on exchange of information for documentation officers from almost all European countries, which took place in Radzikow, Poland, in October 1984. In 1985 some of the databases presented first editions of European forage catalogues. The catalogues varied in format, number of descriptors used and different descriptor states. In this situation, precise definition of format and descriptors for use in European forage lists was needed.

In October 1985, the second meeting of the ECP/GR Forage Working Group decided to extend the descriptor list already used and accepted a greater number of descriptors with precisely defined states. During 1986 the ECP/GR Secretariat, in consultation with all European Forage Data Bases, established a new format for presentation of data accumulated in databases in the form of lists (catalogues). Genetic material presented in a catalogue was divided in general into two parts - breeding material (cultivars, local varieties, mutants, etc.) and collected material. Principles of classification of the material (sorting order of descriptors) in appropriate lists were given. The value of some descriptors was precise. A list of almost all European institutes involved in genetic resources activities has been compiled to enable donor names, collecting institutes and genebanks to be coded using acronyms.

The list consists of about 2 000 institutions in Europe with their full names and addresses. Almost all European country curators and documentation officers took part in the preparation of this list of acronyms.

In the years 1987 and 1988 all European Forage Data Bases presented and distributed within Europe catalogues with passport data in accordance with the standardized format.

2. Utilization of standardized European forage lists

The standardized format of European forage catalogues allows easier understanding of presented data.

The first part of a catalogue, which presents data on bred material, enables an analysis of how far cultivars (or in general "named accessions") are duplicated for safety in other genebanks. On average, 50% of this kind of material is duplicated somewhere in Europe. To ensure full security of the European material it is necessary to have all bred material duplicated in at least one place. Institutes responsible for a database for a particular species should ask all other genebanks for seed of non-duplicated accessions and store them.

The task of selection of accessions could be given to the curators of collections within the institute responsible for the database.

The second part of a catalogue, which contains collected material (ecotypes, wild material, landraces), has been arranged by country of origin and region for the same botanical classification. This enables synthetic presentation of European material in order of geographical distribution for one species.

A catalogue example: the geographical distribution of Festuca:

Festuca arundinacea

nk.*	280
BEL	5
CHE	21
CSK	1
DDR	15
DEU	8
DNK	3
ESP	9
FRA	46
GBR	1
GRC	4
HUN	46
IRL	5
ITA	22
POL	235
ROM	10

Festuca pratensis

nk.	122	NOR	36
AUS	1	POL	241
BEL	1	ROM	20
BGR	1	SUN	11
CHE	22	SWE	1
CSK	10	TUR	10
DDR	10	YUG	1
DEU	93		
DNK	1		
FIN	2		
FRA	18		
GBR	2		
GRC	1		
HUN	32		
IRL	17		
ITA	1		

Festuca ovina

nk.	2
DEU	3
GBR	3
ITA	7
SUN	1
TUR	2

Festuca rubra

nk.	8	IRL	8
DDR	1	ITA	3
GBR	6	NOR	4
GRC	5	SWE	1
HUN	22	WEB	1

and of Dactylis:

Dactylis glomerata

nk.	590	HUN	82
AUT	4	IRL	61
BEL	9	IRN	2
BGR	1	ITA	177
CHE	64	LBY	1
CSK	6	MLT	1
DDR	57	NOR	8
DEU	221	NZL	2
DNK	6	POL	507
DZA	1	PRT	2
ESP	209	ROM	3
FIN	9	SUN	5
FRA	60	SWE	10
GBR	9	TUR	170
GRC	10	YUG	4

Dactylis aschersoniana

nk.	3
GRC	1
ITA	17
LBY	1

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* nk = not known

A similar presentation is possible for all other forage collections and should be carried out so that gaps in collections for some regions (countries) can be identified. The Mediterranean countries could be excluded from the analysis because such an analysis has been carried out in an IBPGR research project on forages of the Mediterranean.

Another way to classify accessions where status of sample is known could be to divide them into wild and cultivated material.

It is important for collectors and curators to include in the standardized list descriptors describing place of sampling. Environmental data from the collecting site enables the collector or curator to suggest possible areas of adaptation in the recipient country and, of course, recording of the location data allows recollection if required.

A taxonomic problem that is commonly found in cultivated plants is becoming more apparent with forage species. There may be no clearly definable discontinuity within a species, or even between species, and there are thus no grounds for the separation of species and species forms. This poses very real problems in communication. The solution is the adoption of only one descriptor for intra-specific taxa if considered necessary or relevant by the respective database.

3. Use of management descriptors for maintenance of collection

Only a small number of management descriptors have been included in the standardized European forage lists. They relate to the regeneration of material (method of regeneration, number of times accession has been regenerated, year of last regeneration and country of regeneration).

Accessions require regeneration as a result of low viability or insufficient number of seeds. It is necessary to consider the possible alternatives, since regeneration is a costly and risky procedure. The first step is to check if the accession has been duplicated in another genebank for safe keeping. A check of the first part of the European standardized lists might be helpful here. The next step, having taken the decision to regenerate, is to consult the primary evaluation data to determine the environment, pollination and isolation requirements for regeneration. Any

information on seed health and insect infestation should also be consulted to ascertain whether any diseases or pests might inadvertently be introduced to the regeneration site during regeneration. Care must be taken not to exhaust seed stocks to enable further attempts at regeneration if the first attempt fails.

Much of the data relating to the regeneration procedure and maintenance are of value only to a genebank. However, some data, such as those relating to the history of a sample as well as to regeneration methods, are of interest to others, especially users.

In this context, the management descriptors included in European lists are adequate and helpful for maintenance of a collection in a particular genebank. Another problem is how far databases should become filled up with this type of information.

APPENDIX V (continued)

ACCURATE IDENTIFICATION OF WILD FORAGE SPECIES by N. Maxted and F.A. Bisby, Dept. of Biology, The University, Southampton, UK

SUMMARY

Poorly identified and/or mixed germplasm collections of wild forage species are of limited value, unless they are correctly identified. The process of identifying seed collections is time consuming and expensive. A prophylactic approach is suggested, in which good collecting practices are used, the material is identified in the field and mixed seed collections are avoided. However, ways of identifying misidentified or unidentified germplasm using seed keys, growing out seed samples, voucher specimen identification and cytotypic identification are discussed.

Introduction

An important aim of genetic resources collection is to collect accurately identified germplasm to represent the range of biological diversity. When a request for germplasm is made to a genebank for accessions of a wild species, the person making the request wants seed of a particular taxon and the only way the genebank can supply this material is if the material held in the genebank is accurately identified.

We do however know of at least one instance where collectors have deliberately made a mixed collection from a mixed crop of forage legumes. The collectors identified the component elements, but retained the collection as a mixture because they wanted to maintain the ratios of the component elements within the crop.

If requests to genebanks require correctly identified seed, how best can this be achieved? The primary approach should be a prophylactic one; both to identify material accurately at the time of collection and where possible avoid collecting mixtures of seed. In general, it is easier to avoid mixed seed collections than to undertake the difficult and costly process of distinguishing separate entities within a mixed collection during the germplasm conservation.

However, as any plant collector knows from experience, adhering to a desirable collection procedure is not always possible. If no field identification is made, or if seed of more than one species is collected under one accession number, then the process of genetic conservation must involve the separation of taxonomic entities followed by the accurate identification of each component.

The following discussion is divided into two, firstly a summary of good collecting practice that avoids the collection of unidentified or mixed accessions, and secondly a discussion of how the problem of unidentified or mixed collection can be resolved. The general emphasis of this paper is toward forage germplasm collection, but the paper is illustrated with specific examples from the legume tribe Vicieae, with which we are both familiar as collectors.

Collecting accurately identified germplasm

Good collecting practice has been extensively reviewed by Hawkes (1980), Ford-Lloyd and Jackson (1986) and Tyler et al. (1987) and so a detailed account will not be reiterated here. In the following we highlight elements of good collecting practice, which relate particularly to the accurate identification of wild forage samples.

Target taxon

The first decision to be made when undertaking a collecting mission is on what taxon the mission will focus. All collecting missions should have a primary target taxon, though this does not preclude the collection of secondary taxa when they are encountered. The choice of target taxon will be either dictated by the expertise of the collecting team or the result of a request from the funding agency.

It is our opinion that a focused mission will more frequently yield the most valuable germplasm of a particular variation pattern: the rare or newly discovered species, or the most sought-after variants within a widespread species. General collections may seem economically effective and provide a large number of accessions, but these collections will be dominated by both common and/or unidentified material.

Target area

The target area will be limited by the choice of target taxon. The area to be visited must have the appropriate geographical and geological conditions to allow the target taxon to grow. The precise target area will be selected on several criteria, such as concentration of target taxon, previous coverage of this area, ease with which the area can be collected (political or geographical limitations).

Collecting Team

The collecting team should be selected so that it includes expertise in identification of the target taxon, expertise in the flora of the target area and expertise in local languages. Many collecting missions in fact comprise two elements: international experts and local counterparts. The former can supply the expert skills at identifying the target taxon and the latter can supply knowledge of the local flora, geography and languages.

A good collecting mission should involve an element of training. The combination of experts in particular plant groups and other botanists provides an ideal opportunity for all the team to broaden their knowledge of less familiar plant groups. Practical experience using keys and descriptions with expert guidance is often the best way for a botanist to familiarize himself with fresh plant groups.

Equipment

The detailed equipment requirements of a collecting mission will be dictated by consideration of the target taxon and area, e.g. whether collecting seed or tubers, whether the team will camp out or stay in hotels. A detailed discussion of these points is provided in Hawkes (1980). For the purposes of collecting at least tentatively identified germplasm appropriate Flora accounts, revisions/monographs of the target taxon and a botanical glossary are essential. Which Flora to use in the target area can be identified using two standard lists of Regional Floras, Frodin (1984) and IUCN (1986). For the purposes of a forage collection recent complete floral accounts (if they exist) of the target area for both the Leguminosae, the Gramineae and other forage groups should be included. For areas where there is no adequate Flora, it may be possible to make use of the Flora of a neighbouring region.

Thus for example the Flora of Turkey lists many of the species found in Syria. The collecting team should take advantage of any other sources of ecogeographic data (e.g. IBPGR ecogeographic surveys) that are available for the target taxon, as these will enable them to locate the desired plant populations more easily.

Major revisions and monographs, which contain distributional and ecogeographic information, may be needed to help locate the target taxon, and keys and descriptions may also be needed to aid specific identification of the material once it is located. Rare forms may not have been seen before, even by the expert, and so some means of providing confirmation of what has been located is needed in the field.

Double site visits

The difficulty in avoiding mixed seed collecting of wild forages germplasm is accentuated in the legume tribe Vicieae. One finds several species entangled together remarkably frequently, Vicia lutea and V. sativa being the commonest example. At seed maturity the crisp shattering plants and pods, apart from making poor herbarium voucher specimens, are neither easily distinguished nor disentangled. Experience of collecting forage legumes in European and Mediterranean areas has shown that a better method is to visit and mark sites beforehand while plants are still green and in flower. This initial visit enables accurate identification of the material found at the site, selection of pure stands, the making of high-quality voucher herbarium specimens and the collection of rhizobia for legume species. We have also discovered that this helps collectors develop a "search image" for the target taxon and thus locate more populations and more species. This is because inconspicuous forms are much more visible when green and flowering than when crisp and brown.

Visiting the collection sites twice also enables the accurate identification of those intra-specific variants, such as the subspecies of Vicia sativa, where both flowers and mature pods are needed for accurate identification. During the second visit to the sites the seed will be collected from the populations identified previously and other populations missed during the first visit will be identified and collected.

Double site visits also enable a better estimate of the fruiting time of that population that year. If seed collection is the primary aim of genetic resources collection, then arrival at the site at the optimum time to collect the largest proportion of seed is important and this optimum time can be more easily estimated if the site has been previously visited that year.

Material collection

Commonly the material gathered during the course of a forage germplasm collecting mission is of five kinds: passport data, voucher specimens, rhizobia, vegetative plants and seed.

The importance of collecting clear, accurate passport data cannot be over-emphasized. This information is vital if particular populations are to be relocated or accessions with particular characteristics identified. Detailed, accurate passport data greatly enhances the value of the genetic resources collected. Over years of experience collecting forages our collecting form has evolved into the form detailed at the end of this paper. This collecting form can be duplicated and held in the field in loose-leaf binders. The sheets should be filled using a pencil as this can be easily erased and does not smudge if collecting conditions are wet. The form is composed of two elements, information concerning the accession collected at that site and information providing details of the site location and site ecogeography. All accessions collected must be given a unique accession number and at least a tentative field identification. The more complete the passport data collected the more useful the germplasm accession will prove to future workers who wish to use it.

Time should always be made available for the collecting of good-quality voucher specimens. Representative flowering and, if possible, fruiting specimens should be pressed. Newspaper is almost as good for this as proprietary drying paper, but flimsy sheets should still be used within the drying paper/newspaper. Newspaper has the added advantage of being easily available and reasonably cheap to purchase. Specimens should be given a field identification and accession number. These should be written on an alpha (jeweller's) tag and attached to the specimen. It is also useful to write the accession number on the flimsy paper, which encloses the specimen inside the drying papers.

If at a later stage any query arises over the identification of a particular accession, the voucher specimen can be consulted and a new identification made for the accession, if appropriate. For this reason voucher specimens should be carefully preserved, i.e. specimens should be pressed firmly, pressing papers should be changed as required, but specimens once placed in a flimsy should remain in it until mounting the specimen on card.

Rhizobia cultures should be taken if forage legumes are being collected. The nodules plus the root fragments should be placed in the culture vial with an alpha tag bearing the field identification and accession number of the plant population. There are very few rhizobia cultures of wild legumes and so it would be useful if the collection of rhizobia from wild legumes were made standard practice. These collections should then be incorporated in the international collections.

With certain pasture forage species (Lolium, Trifolium) vegetative material is collected. This requires that collectors are very familiar with their material and have the appropriate keys to plants in the vegetative state. Tyler et al. (1987) provide details of how to collect and identify vegetative samples. Tyler (pers. comm.) comments that identifying different taxa within the Lolium perenne/L. multiflorum/Festuca pratensis/F. arundinacea complex is particularly difficult during vegetative sampling. On return to a base institution each divot must be separated into a single vegetative unit, grown up and mixed components weeded out prior to anthesis. Tyler also comments that material of this complex is virtually impossible to identify if collected as mixed seed samples.

Care should be taken in collecting seed from populations that have been identified. Seed of any dubiously identified or intra-specific variant plants should be collected under a different accession number. Each seed accession should be placed in a separate bag (paper or cotton, depending on the size of the collection). An alpha tag with the field identification and accession number should be placed inside the collecting bag and these details plus the site number placed on the outside of the bag. It is useful to place all the accessions collected at a particular site inside one larger bag with the site number prominently marked on the outside of the bag.

Providing a name for field populations

Making the field identification of a plant population involves two steps: the detective work of deciding which species the population in the field belongs to, and deciding which name to use for it if there is more than one name in use for that taxon.

The initial detective work is not always as simple as using a key. If the best key available is for an adjacent area it may not include the taxon you wish to identify, or it may be that not all leads in the key can be used, say because pods are absent. In this case eliminating various possibilities may depend on reading descriptions, examining distribution maps or comparing illustrations.

Once the taxon has been identified, there is sometimes the difficulty of synonymity, i.e. the same taxon may be referred to in different books by different names. There is at present no single 'correct' set of names to use, but a good collector should be consistent and use a single system of names for the material collected. The collector may follow the system used in Flora Europaea, Flora of Turkey or MedChecklist, or perhaps in the future one of the taxonomic databases, such as ILDIS, the International Legume Database and Information Service. If collectors are inconsistent and use different naming systems, the identification of their collections will be confusing and ambiguous.

Material conservation

The materials collected require conservation and storage. The data sheets contain two basic kinds of information, on sites and accessions, that can be easily transferred to a simple database comprising two files, one for each kind of data. The files are linked in a relational manner by the site location number. Once the data has been transferred accurately from the data sheets to the database, the physical collection can be easily managed and conserved.

The dried voucher specimen should be reidentified on return to the laboratory to check the field identification. This laboratory identification should also be entered into the database. The voucher specimens can then be mounted on card and the herbarium label produced from the database automatically, saving time and avoiding errors in typing repetitive information.

The rhizobia can be cultured or sent to the appropriate international collection for culturing. As with the voucher specimens, labels for the culture can be printed automatically from the database.

The seed collections should be fumigated (if required), threshed, cleaned, divided (if required) and dried as soon as possible after collection, to avoid any rapid deterioration in quality of the sample. Each time the seed sample is transferred from one container to another there is potential for error from mixing the accession, so it is advisable to make the minimum number of container changes from the original bag to its genebank seed container. Avoid having the bags of several accessions open at any one time. It is better to thresh one accession at a time and so be sure of avoiding mixing of samples. Labels for the genebank seed containers can also be produced direct from the database.

Identifying germplasm at the conservation stage

Despite these general rules it must reluctantly be admitted that even the best collecting teams occasionally gather mixed collections or are unable to identify a particular population of plants. Both mixed and unidentified accessions are of little value, unless steps are taken to separate and/or identify them. Resolving this problem involves two distinct tasks: sorting the seed into the component species, and identifying the component species.

Sorting individual components from mixed accessions

Sorting out the component species in a mixture is a task that varies enormously in complexity. It can be trivially easy if the seeds fall into two or three distinct classes, especially if the classes are both distinct in colour, size, hilum or ornamentation and each class is relatively uniform. Vicia faba and V. narbonensis, for instance, are grown in a forage mixture in the eastern Mediterranean. Seed mixtures of these two species are easily separated because of their distinctive seed shape, size and colour. The problems arise either if the components are not visually distinct, or if one or other of the components is very variable. Examples of high levels of variation occur if a component species shows well developed polymorphisms of seed size or coloration.

Once the components are separated, identification can be undertaken either using seed identification keys, or by germinating samples and identifying the growing plants.

Using seed keys

Many species can be distinguished by microscopic examination and the use of keys specifically written for identifying seeds. Examples for the Vicieae are: Zertova (1962); Leokene (1966); Gunn (1970, 1971); Voronchikhin (1981); Lersten and Gunn (1982); and Perrino et al. (1984). The process of identifying individual seeds from a mixed collection is very time-consuming, tedious and will only work for the more remotely related species. Most wild genetic resources collection missions will have as their target taxon close relatives of one or more crop plants and the relatives of each crop will in turn be commonly closely related to each other, and so, almost by definition, difficult to distinguish using seed keys.

It may seem an obvious point, but if the mixed collection cannot be separated visually into its components, it is of little help to learn that the original collection contains Species A and Species B. The original sample will remain useless, but individual seeds, grown out and identified, will produce progeny seed that is of value to a genetic resources collection.

Growing out

If the seed can be germinated, grown into a plant and brought successfully into flower and fruit, then the process of identification can begin, using the relatively easily distinguishable flowers and/or fruits characters. An additional bonus of growing out mixed accessions is that flowering or fruiting voucher specimens can be taken for each of the components of the mixture. One voucher specimen cannot represent the entire mixed collection.

During a forage legume collection mission to Syria in 1986, Vicieae material from the Genetic Resources Unit, ICARDA was grown out for identification checking. In all, 2 528 accessions were planted in individual plots. As the plots came into flower and pod, so accessions and components of mixed accessions were identified by Vicieae specialists from the University of Southampton.

For the mixed accessions individual plants were identified and labelled with alpha tags. Voucher specimens were taken for all accessions at the flowering stage. At fruition, seed from each plot or plot component was collected into a suitably labelled bag ready for conservation. Fifty-eight species were identified from the ICARDA collection and about 250 labelled as Vicia sp., Lathyrus sp. or Lens sp. were fully identified. It should be reiterated that this process is labour intensive, but it does have the secondary benefit of allowing the bulking up of the grown-out samples.

It should be noted however that the growing out of wild collections in plots does have inherent problems. The ecogeographic conditions of the plots will place selection pressure on the accessions for those particular conditions. Also, if the species are outcrossing, the close proximity of each plot will facilitate cross pollination. This is not a problem for the self-pollinating annual Vicieae, but may be much more of a problem in other groups, necessitating bagging of plants.

Voucher specimen identification

If the collecting team cannot identify a population in the field or is unsure of the identification, the collection can be identified at a later date using the voucher specimen. There is however the risk that the collector may have taken a voucher specimen that does not represent the collected material or that the collection may be mixed, so that the voucher will only enable one component of the mixture to be identified.

During the joint Turkish Ministry of Agriculture and University of Southampton forage legume collecting mission to Turkey in 1987, voucher specimen identification was used to identify over 700 Vicieae voucher specimens in the herbarium at the Aegean Agricultural Research Institute, Izmir. A sizeable proportion of the voucher specimens were either mis- identified or unidentified and so the accurate identification of these specimens and their accompanying germplasm greatly enhanced the collection's value.

Cytotypic identification

Special problems are encountered when collecting taxa that are virtually impossible to identify in the field and where the only sure identification can be carried out in the laboratory. An example of this is the sympatric populations of Festuca pratensis ssp. pratensis and ssp. apennina, where certain spikelet characters can be used as a rough identification, but the only way to identify the two subspecies positively is to count the chromosomes (ssp. pratensis, 2n = 14; ssp. apennina, 2n = 28). Another example is encountered with collecting the diploid and hexaploid forms of Dactylis glomerata.

Discussion

There will always be those germplasm collectors that collect large quantities of mixed accessions, or numerous Trifolium spp., Lolium spp. or even legume species; but this is wasteful of time and resources. Material collected as 'Vicia' sp. or 'Lolium' sp., unless passed on to experts with adequate resources, is likely to remain useless. So it is generally much better to avoid mixed or unidentified collections, if at all possible. If, however, mixed or unidentified collections are made, there are ways of separating the mixed components or identifying the unknown seed sample.

Acknowledgements

We appreciate the assistance of Dr. B.F. Tyler for providing specific examples of identification problems from the Gramineae.

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IBPGR COLLECTION OF WILD FORAGE GERMPLASM

Country ..... Province ..... Date.../.../88

Site Number ..... Nearest Village..... .....

Location .....

Altitude(m).....Latitude.....N Longitude.....E Rainfall.....cm

Site Physical .....

Site Vegetative .....

Coded Environmental Information:

PR	TS	pH	ST	AS	SL	%C	DS	WR	AP	GP	%R	RT	%T	TT	PN
/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/

Taxon Accession Information:

Coll. Nos ..... Name .....

Petal Colour Standard ..... Wing ..... Keel .....

Habitat ..... Pop. Character .....

Herb. Spec. Y/N Nos. Duplic ..... Date .../.../88 Rhizobia Y/N

Seed Coll. Y/N Coll. Size ..... Nos. Plants Sampled .....

Date of Seed Coll. 1 .../.../88 2 .../.../88 3 .../.../88

Coll. Nos ..... Name .....

Petal Colour Standard ..... Wing ..... Keel .....

Habitat ..... Pop. Character .....

Herb. Spec. Y/N Nos. Duplic ..... Date .../.../88 Rhizobia Y/N

Seed Coll. Y/N Coll. Size ..... Nos. Plants Sampled .....

Date of Seed Coll. 1 .../.../88 2 .../.../88 3 .../.../88

Coll. Nos ..... Name .....

Petal Colour Standard ..... Wing ..... Keel .....

Habitat ..... Pop. Character .....

Herb. Spec. Y/N Nos. Duplic ..... Date .../.../88 Rhizobia Y/N

Seed Coll. Y/N Coll. Size ..... Nos. Plants Sampled .....

Date Of Seed Coll. 1 .../.../88 2 .../.../88 3 .../.../88

ENVIRONMENTAL DATA CODES

Parent Rock (PR)

A = Peat and coal
B = Conglomerate
C = Sandstone
D = Sahles - mudstone
I = Siliceous
J = Limestone
K = Laterites
L = Granites
M = Dolerites
N = Phyolites
O = Basalts
P = Hornlels
Q = Slate
R = Schist
S = Quartzites
T = Alluvium
U = Dunes

Type Of Soil (TS)

A = Calcic brown
B = Terra rossa
C = Heavy black
D = Woodland brown
E = Alluvial
F = Sandy loam
G = Clay

Slope (SL)

L = Level 0-3%
U = Undulating 3-8%
R = Rolling 8-16%
M= Moderate 16-30%
S = Steep > 30%

Depth Soil (DS)

A = 0 -10cm
B = 10-20cm
C = 20-40cm
D = > 40cm

Water Relations (WR)

F = Free draining
R = Run-off
S = Swamp

Agricultural Practice (AP)

S = Pasture
A = Fallow
C = Crop
G = Grassland
F = Forest
W = Woodland
R = Roadside
P = Protected enclosure
D = Disturbed

Grazing Pressure (GP)

A = Nil
B = Light
C = Moderate
D = Severe

Rock Type (RT)

A = Flat
B = Rocks
C = Boulders
D = Large boulders

pH = Estimate of soil acidity
AS = Aspect
%C = % site with ground cover
%R = % site covered by rocky outcrops
%T = % site covered by trees & shrubs
PN = Photograph number

Soil Texture (T)

G = Gravel
S = Sand
Y = Sandy loam
L = Loam
M = Clay loam
C = Clay

Type of Tree Or Shrub (TT)

1 = Shrubs
2 = Small trees
3 = Medium trees
4 = Large trees

PRE-BREEDING IN GENETIC RESOURCES OF PERENNIAL RYEGRASS (LOLIUM PERENNE L.) by Chr. Paul, Institut für Grünland- und Futterpflanzenforschung der Bundesforschungsanstalt für Landwirtschaft (FAL), Braunschweig, FRG

ABSTRACT

The first phase of an ongoing project is described which aims at developing perennial ryegrass populations with suitable agronomic features to justify their further development by commercial breeding. It is shown how a system of reference varieties was used for standardizing heading dates of 702 genebank accessions distributed over 11 West German sites for testing. Besides heading date, winter survival and vigour were also assessed on 40 single plants of each accession over two harvest years. Selection for vigour within maturity classes resulted in nine newly formed populations that will be subjected to further testing under sward conditions in the following phase of the project.

1. Introduction

After the storage and documentation of genetic resources as base collections (for definition see Frankel, 1975) the next major task for genebank curators will be to set up a system that enables plant breeders to request accessions with specific attributes. This purpose would be served by an active or working collection organized in a user-friendly way. Plant breeders expect that such working collections are able to provide a variety of accessions with acceptable levels of productivity under actual farming practice. The theoretical options for structuring working collections are:

- to maintain the genetic identity of accessions by providing duplicates from base collections;
- to broaden the genetic identity of accessions by providing bulked/pooled accessions; or
- to change the genetic identity of accessions by selection according to the needs of agricultural practice.

It is obvious from the large number of accessions in many genebanks that performance testing during evaluation cannot be carried out with the same reliability as in official variety tests. Multisite trials over a number of years only become feasible when the number of entries are reduced, as can for instance be achieved by bulking (see P. Guy et al., Appendix V). After such a step has been taken it is logical to proceed by subjecting these bulks to selection - now called pre-breeding in this context - in order to produce attractive populations for the plant breeder.

In the Federal Republic of Germany these considerations were used as guidelines for handling germplasm of perennial ryegrass after spells of cold weather in 1981/82 had caused considerable winter kill with ensuing yield losses of that crop. It was then felt that the available perennial ryegrass varieties should be supplemented by material more adapted to continental winters.

The results presented below were obtained during a project that was set up to screen an entire working collection of perennial ryegrass from a continental background for broad adaptation and vigour as spaced plants (first phase), to form new populations from selected plants in discrete maturity groups (second phase) and to test the performance of the newly formed populations under sward conditions (third phase). The work during the first phase (reported here) was only possible through the participation of several German grass breeding companies as well as university and government research institutes.

2. Materials and methods

Perennial ryegrass accessions (n = 708) for testing and evaluation were kindly provided by the Plant Breeding and Acclimatization Institute at Radzikow, Poland, from the base collection held at that institute. The material consisted of accessions in the form of ecotypes or bred varieties mainly of eastern European origin. For reference purposes the perennial ryegrass varieties given in Table 1 were used.

TABLE 1. **Perennial ryegrass varieties for reference purposes**

Variety	Heading date[1/,2/]	Maturity class[2/]
Gremie	47	Very early
Liprior	53	Very early - early
Hübal	60	Early - medium
Morenne	60	Early - medium
Kerem	61	Medium
Lihersa	66	Medium - late
Parcour	69	Late
Perma-	69	Late
Vigor	72	Late - very late
Donata	78	Very late

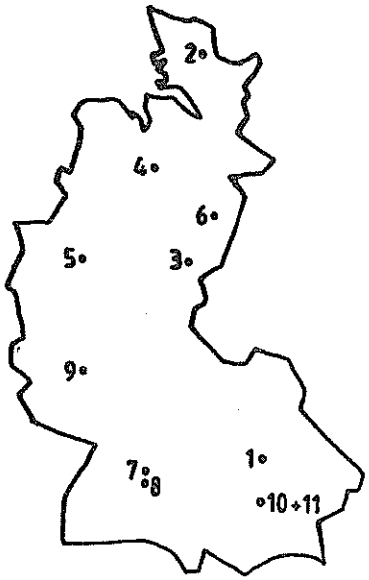
1/ Number of days after 1 April

2/ Characterization by Federal Variety Testing Office

The procedure for testing and pre-breeding comprised the following steps:

1. Formation of 11 test sets of 50 or 100 accessions each and dispatch of one test set and the above reference set to each of the 11 participating institutions (some of the test sets included accessions also appearing in one or several other test sets).

2. Establishment of 40 single plants per accession at the participating institutions in the summer of 1985 (see geographical location of sites in Figure 1).



1 = Steinach
2 = NPZ
3 = Göttingen
4 = Asendorf
5 = Lohne
6 = Völkenrode
7 = Hohenheim
8 = Oberer Lindenhof
9 = Oberdielbach
10 = TU Freising
11 = Bayerische Landesanstalt Freising

Figure 1. Geographical location of test sites

3. Visual assessment of single plants under an infrequent cutting system (two harvest cuts) in the first harvest year (1986) for winter survival, heading date and vigour.

4. Visual assessment of single plants under a frequent cutting system (a minimum of three harvest cuts) in the second harvest year (1987) for winter survival and vigour.

5. Compilation of heading dates of reference varieties across sites and regression analysis for allocation of each single plant to site-corrected maturity classes.

6. Compilation of the data of individual test plants and their ranking for average vigour in site-corrected maturity classes.

7. Selection of superior plants and formation of nine new populations (one population per maturity class).

3. Results

3.1 Heading date across sites

Following the decision that each tested single plant had to be allocated to its appropriate maturity class irrespective of where it was grown, the reference varieties were used to define prediction formulas for this purpose.

Mean heading date of all single plants per reference variety was calculated for each variety and site. Also, mean heading date across sites was calculated for each variety. On this basis, regression functions could be established for predicting the mean date of heading across sites of any variety from its heading date at any single site (see Figure 2). The difference between the extreme regressions indicated that the spread in heading dates between the earliest site (Steinach) and the latest site (Oberer Lindenhof) corresponded to an interval of two weeks.

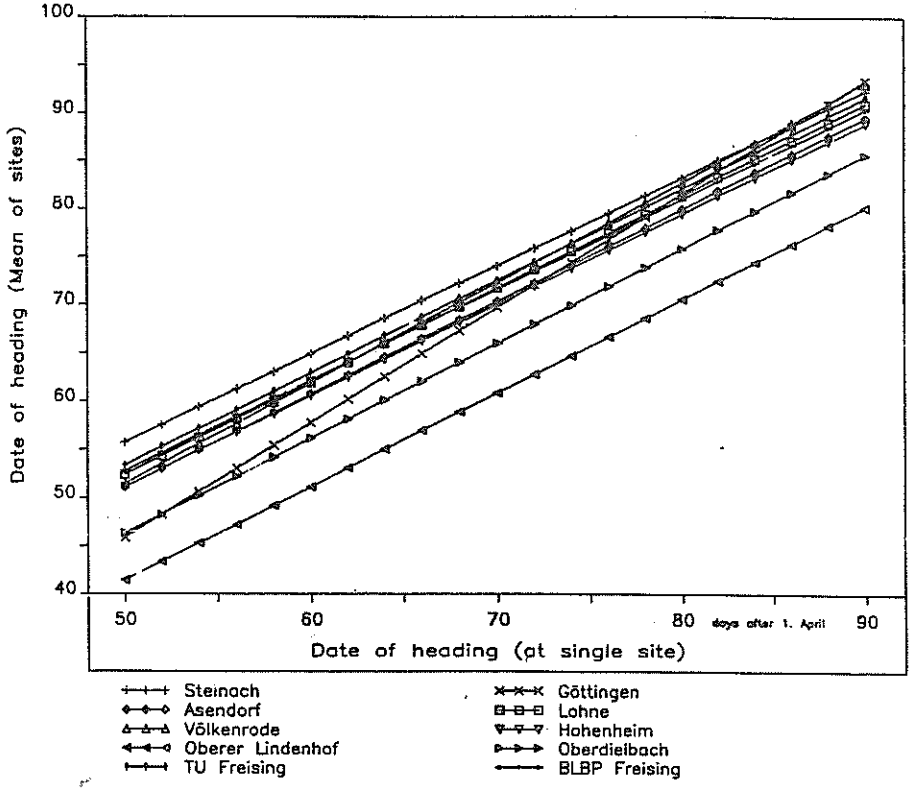


Figure 2. Site-specific correction functions for date of heading based on reference varieties

The only site showing interactions for heading date was Göttingen. In all cases close fits were observed as can be seen from the fact that the coefficient of determination (r^2) always exceeded 0.96 for the relationships shown.

Regression functions between heading dates at any two given sites were also calculated. Thus the heading date of an accession at a given site 'x' could be used for predicting its heading date at site 'y'. Since some accessions had been included in two or more test sets and had thus been assessed at two or more sites the regression functions could be subjected to independent validation. The results given in Figure 3 show a close fit for 92 comparisons of predicted versus observed (r = +0.95). Taken together, the observations on heading dates of reference varieties across sites provide a reliable data basis for prediction at a site even if actual observations are only available for another site.

3.2 Selection of test plants after allocation to discrete maturity classes

The above system of regression functions permitted the calculation of heading dates corrected for site effects. In this way, nine maturity classes were formed. The relative frequency of plants in these nine maturity classes within sites can be seen in Figure 4. Apparent deviations from a normal distribution were evident at sites where only 2 000 plants (50 accessions x 40 plants) had been tested (e.g. Oberdielbach, Lohne, Göttingen).

For each maturity class a maximum number of 100 single plants with superior vigour over successive cuts was selected. Each site contributed to these as many plants as corresponded to its relative proportion of total plants within a maturity class. This was intended to minimize possible imbalances that might have arisen through site-specific scaling effects in the visual assessment of vigour.

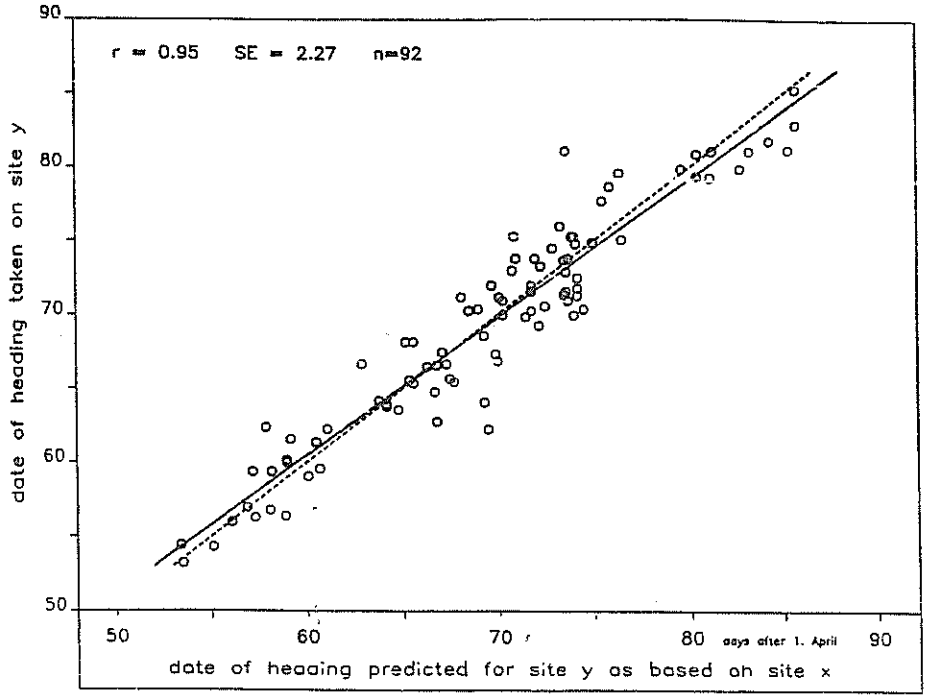


Figure 3. Relationship between observed and predicted date of heading for unknown ecotypes

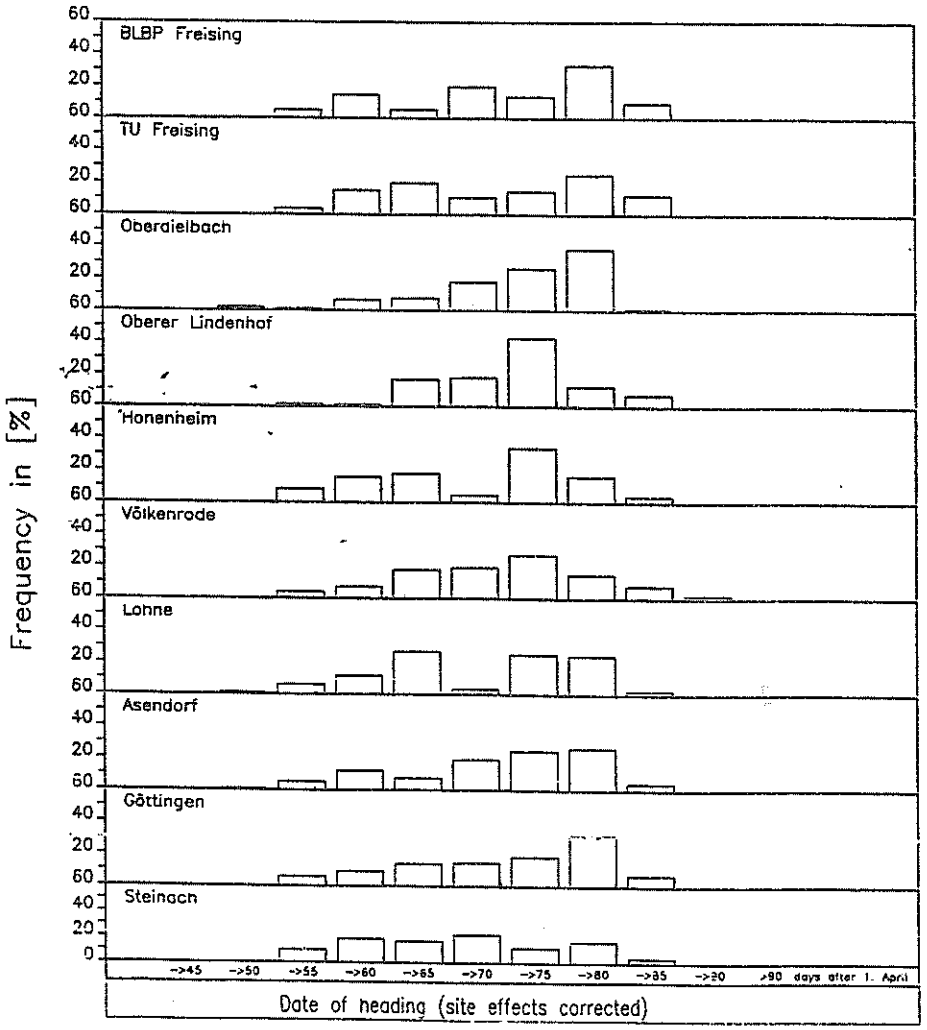


Figure 4. Frequency of plants at given heading dates within test sites (ecotypes grouped according to maturity)

The distribution of total and selected plants across maturity classes summed over sites (Figure 5) demonstrates the tremendous difference in selection intensity in the different maturity classes. On the one hand, the class with the same expression of heading date as the variety Parcour (between 75 and 80 days after 1 April) comprised almost 6 500 plants in total of which the 100 selected plants formed only a 1.5% portion. On the other hand, no selection was applied in the extremely early and late maturity class

4. Discussion

Conceptually the above project draws upon perennial ryegrass populations from continental origins and restructures them by pre-breeding. It incorporates an evaluation system like that recommended by IBPGR/CEC (1985) which moves from a preliminary to a further evaluation. However, the methodological approach used here clearly deviates from that necessary to describe base collections of genetic resources. It must be assumed that the way in which the original perennial ryegrass accessions were screened for heading date allowed relatively precise phenological characterization. The high heritability for heading date will ensure additionally that the nine newly formed populations remain discretely different in maturity in the following generation.

Selection among single plants such as the one practised here for vigour has often attracted criticism because, in addition to low heritabilities of performance characteristics on a single plant basis, low repeatability between single plant and sward conditions appears to have led to faulty conclusions (e.g. Tyler and Jones, 1982). On the other hand, it has also been found that differentiation between ill- and well-adapted forms of perennial ryegrass is equally clear under single plant and sward conditions (Paul, unpublished).

A plausible explanation for such a finding might be that in all cases where a genetic basis for tolerance to biotic and abiotic stress factors exists and varies in expression between populations, phenotypic differences should be measurable and correlated under single plant and sward conditions. This is why selection for productivity characteristics under single plant conditions cannot be discounted in general and might even be advantageous where a large number of accessions with large exploitable variability has to be screened (see e.g. Burton, 1981).

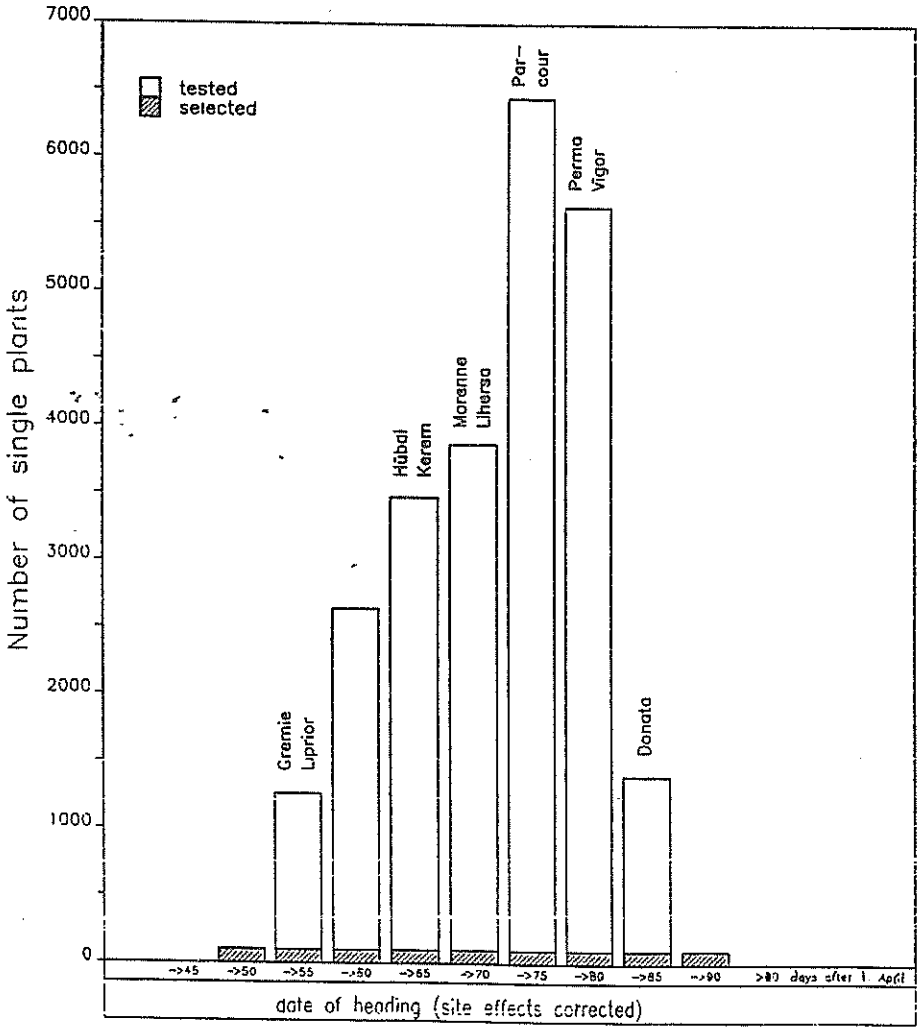


Figure 5. Absolute number of plants (tested and selected) at given heading dates across test sites (heading date of reference varieties indicated by variety name)

In the case of the project considered here, the relatively high selection intensity used among single plants with a near average expression of heading date has a further consequence. Since the heritability of vigour can be assumed to be above zero, the populations founded after polycrossing the selected single plants should exhibit above-average vigour as a response to selection. In contrast, the extremely early and late individuals were not selected for vigour and may have to be subjected to recombination and selection to reach acceptable levels of performance in vigour.

Later on in the project, the populations are to be tested under sward conditions at multiple sites, so it will then be possible to verify the above expectations.

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