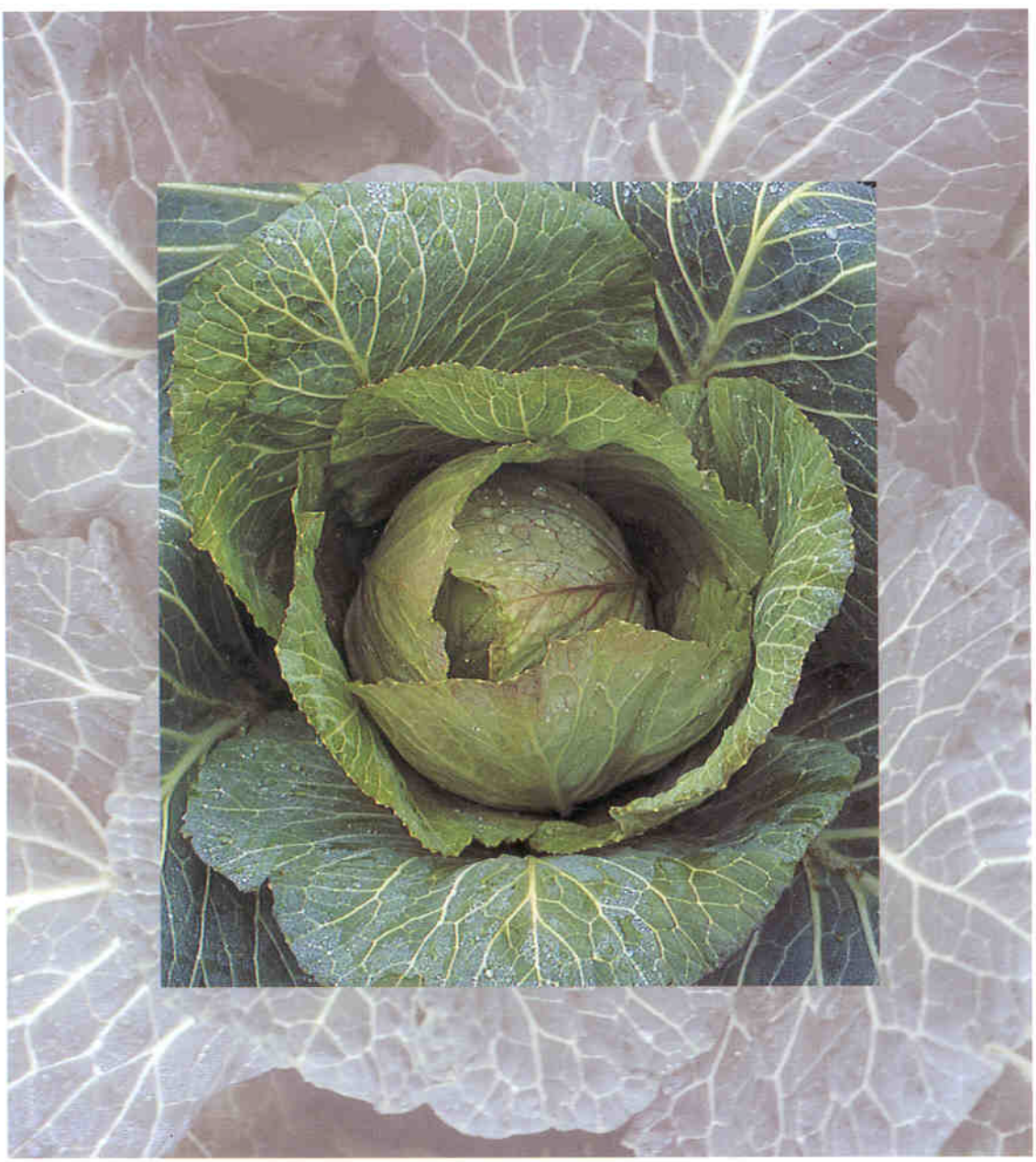
EUROPEAN COOPERATIVE PROGRAMME FOR CROP GENETIC RESOURCES NETWORKS (ECP/GR)

Report of a Working Group on *Brassica*

Third meeting
27-29 November 1996
Rome, Italy

L. Maggioni, D. Astley, M. Gustafsson, T. Gass and E. Lipman, *compilers*



International Plant Genetic Resources Institute
IPGRI

EUROPEAN COOPERATIVE PROGRAMME FOR CROP GENETIC RESOURCES NETWORKS (ECP/GR)

Challenges in rye germplasm conservation

Proceedings of an International Conference on Crop Germplasm Conservation with Special Emphasis on Rye, and an ECP/GR Workshop
2-6 July 1996
Warsaw/Konstancin-Jeziorna, Poland

T. Gass, W. Podyma, J. Puchalski
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International Plant Genetic Resources Institute
IPGRI

The International Plant Genetic Resources Institute (IPGRI) is an autonomous international scientific organization operating under the aegis of the Consultative Group on International Agricultural Research (CGIAR). The international status of IPGRI is conferred under an Establishment Agreement which, by March 1997, had been signed by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mauritania, Morocco, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovak Republic, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine. IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI works in partnership with other organizations, undertaking research, training and the provision of scientific and technical advice and information, and has a particularly strong programme link with the Food and Agriculture Organization of the United Nations. Financial support for the research agenda of IPGRI is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, Finland, France, Germany, India, Italy, Japan, the Republic of Korea, Luxembourg, Mexico, the Netherlands, Norway, the Philippines, Spain, Sweden, Switzerland, the UK and the USA, and by the Asian Development Bank, CTA, European Union, IDRC, IFAD, Interamerican Development Bank, UNDP and the World Bank.

The European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) is a collaborative programme among most European countries aimed at ensuring the long-term conservation and facilitating the increased utilization of plant genetic resources in Europe. The Programme, which is entirely financed by the participating countries and is coordinated by IPGRI, is overseen by a Steering Committee (previously Technical Consultative Committee, TCC) composed of National Coordinators nominated by the participating countries and a number of relevant international bodies. The Programme operates through ten broadly focused networks in which activities are carried out through a number of permanent working groups or through ad hoc actions. The ECP/GR networks deal with either groups of crops (cereals, forages, vegetables, grain legumes, fruit, minor crops, industrial crops and potato) or general themes related to plant genetic resources (documentation and information, *in situ* and on-farm conservation, technical cooperation). Members of the working groups and other scientists from participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme.

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Opening session

Introduction

The third meeting of the *Brassica* Working Group was held from 27 to 29 November 1996 in Rome, Italy. The meeting was attended by 21 participants representing 20 countries, FAO and IPGRI (see List of participants, Appendix I).

The meeting was opened by Thomas Gass, Director of the IPGRI Regional Office for Europe, who welcomed the participants on behalf of the organizers. He emphasized the importance of this second meeting of the Working Group since the beginning of Phase V, which should be considered an opportunity to assess the progress made in implementing the Group's workplan and decide on the necessary steps to ensure a productive Phase V for the Working Group. Thomas Gass then introduced Lorenzo Maggioni, the new ECP/GR Coordinator.

Lorenzo Maggioni welcomed the Group and expressed his pleasure in being able to work closely with the *Brassica* Working Group. With reference to the Group's endeavors to make regional *Brassica* conservation efforts more effective and to address the *in situ* conservation of relevant species, he noted the importance of these issues for ECP/GR considering the responsibility of European countries in contributing towards the conservation of this important indigenous genepool.

Mats Gustafsson, Chair of the Working Group, then asked the participants to briefly introduce themselves. He outlined the primary objective of the meeting which is to follow-up on the workplan established as a result of the 1994 meeting, and to discuss the future activities of the Group. The agenda suggested for the meeting was adopted with minor modifications. The discussions and recommendations are summarized in the first part of the present report. Individual papers are included in the second part in a non-refereed form. The Chair's report of the period since the last meeting also is included in the second part.

Regional cooperation in crop genetic resources: recent events

Thomas Gass gave an overview of the regional preparatory process leading to the International Technical Conference on Plant Genetic Resources (ITC) held in June 1996 in Leipzig, Germany, and explained some of its implications for the ECP/GR *Brassica* Working Group. He described the changes in the structure of ECP/GR decided by the National Coordinators during the Mid-Phase Meeting of the Technical Consultative Committee (TCC) and reminded the Group that regional networks were recognized as key elements in implementing the Global Plan of Action adopted in Leipzig.[1]

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[1] See following references:

Gass, T., G. Kleijer, M. Waldman and E. Frison, editors. 1995. Report of the Technical Consultative Committee. Sixth Meeting, 21-23 September 1995, Nitra, Slovakia. ECP/GR. IPGRI, Rome, Italy.

IPGRI/FAO. 1996. International Technical Conference on Plant Genetic Resources: Preparatory process for Europe. IPGRI/FAO, Rome, Italy.

FAO. 1996. Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture. FAO, Rome, Italy.

A discussion followed during which it was noted that crop genetic resources and the genebanks conserving them have recently received much international attention, but that the financial commitment of governments to long-term conservation is still low in a number of countries. In particular, the cost of certain regional tasks (e.g. central crop database management, chairing of working groups, etc.) carried out as inputs in kind by working groups' members within the framework of ECP/GR are frequently underestimated.

The European *Brassica* Database (Bras-EDB)

Report of the ECP/GR-EGDS[2] documentation meeting

Lorenzo Maggioni summarized the outcomes of the documentation meeting held in October 1996 in Budapest. The consequences for the Bras-EDB concern the standardization of passport data to facilitate data exchange, recommendations regarding the inclusion of characterization and evaluation data into the central crop database to make it more directly useful to breeders, researchers and other users, and the possible future publishing of the database via Internet under the umbrella of the new ECP/GR information platform on crop genetic resources.[3]

During the discussion that followed it was emphasized that the multicrop passport list proposed by FAO/IPGRI should be considered as a standard format for data exchange between genebanks and the central databases. It was noted that the recommendation to include characterization data into the Bras-EDB will be implemented through the set of minimum descriptors agreed upon in 1994 in Lisbon.[4] With regard to evaluation data the Group decided as a first step to include meta-data pointing towards the source of such information.

The Group decided that the Bras-EDB should be included into the proposed Internet platform, but that the services provided in this way will be extended gradually, providing at first the whole database on diskette or in downloadable form and eventually direct for on-line searches. It was confirmed by IPGRI that an advisory group and relevant training workshops will be organized to facilitate the establishment of these services.[5]

Status of the European *Brassica* Database (Bras-EDB)

Ietje Boukema presented the status of the Bras-EDB and gave an overview of the updates carried out since the last meeting (see this volume). She provided each participant with a complete electronic copy of the database to be forwarded to the national genebank managers. The Bras-EDB currently includes records of 12 849 accessions conserved in 21 collections in 17 countries within the region. Duplicate tracing is a continuous, time-consuming activity of the Bras-EDB manager. So far

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2 EGDS: Dutch project for Eastern European Germplasm Documentation Systems

3 Lipman, E., M.W.M. Jongen, Th.J.L. van Hintum, T. Gass and L. Maggioni, compilers. Central Crop Databases: Tools for Plant Genetic Resources Management. 1997. IPGRI, Rome, Italy.

4 Gass, T., M. Gustafsson, D. Astley and E.A.Frison, compilers. 1995 Report of a working group on Brassica (Second meeting, 13-15 November 1994, Lisbon, Portugal). ECP/GR. IPGRI, Rome, Italy.

5 As of January 1997 the Advisory Group has been formally established. The members of the group are Pierre Campo (GEVES), Theo van Hintum (CGN), Morten Hulden (NGB), Daniel Jiménez Krause (ZADI), Kevin Painting (IPGRI) and Lorenzo Maggioni (ECP/GR Coordinator).

1738 putative duplicates have been identified within the database. Ietje Boukema informed the Group that the number of information requests from the database has significantly increased since the last meeting of the Group in Lisbon.

The principal change made to the Bras-EDB since the last meeting of the Group has been the inclusion of the database on wild *Brassica* compiled and managed by UPM, Madrid, as well as data received from Zaragoza and other Spanish collections, and from Belgium.

A completely revised set of data was received from Vratislav Kucera regarding the Czech collections but the changes have not yet been incorporated into the database.

Updated datasets were provided at the meeting for the collections in Bulgaria, Poland, Russia, and Spain (Zaragoza).

Datasets are still missing from Austria, Croatia, Portugal, and the Italian collections maintained in Palermo and Catania.

During the discussion, I. Boukema pointed out that, for a relatively large number of accessions, the taxon and population type are recorded as unknown in the Bras-EDB. She distributed a table indicating numbers of these accessions per country. The meeting agreed that each genebank manager will check the accessions for which taxon or population type are recorded as unknown and confirm to the database manager by July 1997 whether the information is effectively unknown and the accession requires determination. After completion of this first survey, wild or dubious material requiring taxonomic identification will be sent to UPM where they will be grown and determined by César Gómez-Campo.

Regarding the frequency and type of updating of the Bras-EDB, it was agreed that a comprehensive rebuilding of the database may eventually become necessary and could be more efficient than the continuous inclusion of new data. However, in consideration of the numerous updates already prepared and sent to the Bras-EDB and the little time left to the end of ECP/GR Phase V, it was agreed that updates received by the database manager before September 1997 would be incorporated into an updated version of the Bras-EDB to be finalized by May 1998.

It was agreed that characterization data presently available in genebanks in the format agreed for the minimum descriptor list (given in the report of the Lisbon meeting) should also be sent to the Bras-EDB before September 1997 for subsequent inclusion in the May 1998 version. Ietje Boukema will facilitate this process by sending a letter to the genebanks inviting them to contribute characterization data and informing them of the code and structure for the respective descriptors in the Bras-EDB.[6]

The necessity to include availability of accessions as a separate field in the database was discussed. It was agreed that this was not needed since material declared to the Bras-EDB by the genebanks is usually available, although in restricted quantities and for the purposes of breeding or research. It was also agreed that the genebanks should keep clear records of any accession which is discarded or lost. These data will not be centralized in the Bras-EDB.

[6] This letter was sent in July 1997.

Status of *Brassica* genetic resources collections

Status reports not included in the report of the Second Meeting (Lisbon, 1994)[7]

Bulgaria

Stefan Neykov presented the status of the Bulgarian *Brassica* collections. The national base collection is maintained at IIPGR, Sadovo near Plovdiv and contains a total of 622 accessions. Active collections are maintained at the Institute of Vegetable Crops in Maritsa, Govna, Oryahovitsa and Sandansk.

Croatia

Vesna Zidovec provided an overview of the occurrence of wild *Brassica* species in Croatia and outlined national activities aiming at the collection and evaluation of these genetic resources as well as the diversity of cultivated *Brassica* forms in the country. She expressed the strong interest of the Croatian National Plant Genetic Resources Programme to collaborate with other members of the Group in the collection and conservation of Croatia's *Brassica* genetic resources.

Cyprus

Athena Della, who was unable to attend, informed the Group by correspondence that there is no collection of *Brassica* spp. in the Genebank of the Cyprus Agricultural Research Institute apart from a few seeds of the endemic *B. hilarionis* Post obtained from the collecting mission of Mats Gustafsson and César Gómez-Campo in 1986. This is one of the protected species included in the list of the Bern Convention. She also sent information regarding species of *Brassica* recorded in Cyprus which include *B. hilarionis* Post, *B. tournefortii* Gouan, *B. nigra* (L.) Koch and *B. napus*.

Russian Federation

Larisa Shashilova presented the status of the *Brassica* collection conserved at the N.I. Vavilov Institute in St. Petersburg (VIR) and its long-term seed store at Kuban (Krasnodar Region). An important recent activity has been the transfer of the genetic resources collections maintained in St. Petersburg to deep freezers at –18°C. So far, 30% of the collection has been transferred to long-term conditions. The passport data of the collection have been computerized. Larisa Shashilova provided a diskette of these data for the Bras-EDB.

Slovakia

Magdalena Valsiková gave an overview of the *Brassica* conservation activities in Slovakia. She mentioned that during the initial phase of restructuring the national agricultural research system, a number of valuable *Brassica* accessions were lost as a result of privatization of breeding institutes. Recently the Slovak Genebank including modern and long-term conservation facilities was inaugurated at RICP, Piestany. A national project exists to duplicate all germplasm collections held by the country's breeding stations to the base collection in Piestany. An agreement has been signed with RICP, Prague, Czech Republic for reciprocal safety-duplication of the two countries' base collections.

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[7] Only brief highlights of the presentations are given here; full texts of papers are in this volume.

Turkey

S. Ali Küçük presented the *Brassica* conservation activities in Turkey. A base collection complying with internationally accepted standards is maintained at the AARI Genebank in Izmir. Küçük described the distribution of wild *Brassica* in Turkey and summarized the results of missions carried out to collect this diversity.

Updates to status reports received during the Second Meeting (Lisbon, 1994)[8]

Czech Republic

Vratislav Kucera described recent changes in the status of the Czech *Brassica* collections. The genebank of RICP, Prague-Ruzyne maintains the former collection of Olomouc which has been reviewed recently to have an exact account of viable material and to exclude breeding material from the genetic resources collection. Additional material has been added to the collection as a result of collecting missions in Slovakia and Bohemia and germplasm exchange with the Vavilov Institute in Russia. The second collection, held at the Research Institute for Oilseed Crops in Opava has not changed. A new collection of *Brassica* fodder crops is being established at the Research Institute for Fodder Crops in Troubsko, near Brno.

France

Grégoire Thomas informed the Group that no more collecting actions have been undertaken since the last meeting, though cabbage landraces could still be collected in some areas. Emphasis is being set on the regeneration of the *B. oleracea* landraces through bulking procedures (see **Rationalization of collections and related studies of the diversity spectrum**, below).

Germany

Klaus Dehmer presented an overview of the collections maintained at BAZ, Braunschweig, and at IPK, Gatersleben and Malchow, for a total number of 24 species and 4708 accessions. He gave results of germination tests carried out on seeds after different lengths of storage. Morphological, cytological, isozyme and PCR-based analyses are also being carried out on perennial kale (*Brassica oleracea* var. *ramosa)* to identify homogenous classes in view of reducing accession numbers and consequently the work needed for their maintenance.

Greece

Stelios Samaras explained that the status of the Greek *Brassica* collection has not changed since the last meeting. It includes 169 accessions of which 43 are wild species. Priority for collecting missions to be carried out in 1997 is given to the Ionian Islands and the coast of the northwestern mainland.

Italy

Pietro Perrino gave a comprehensive overview of the germplasm maintained at the Germplasm Institute in Bari and indicated some data changes and updates which he will provide to the Bras-EDB manager. The material in Bari is well documented for passport data but is not characterized or evaluated.

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[8] Only brief highlights of the presentations are given here; full texts of papers are in this volume.

Giuseppe Venturella presented the status of the recently established collection in Palermo. This collection focuses on the conservation and study of wild *Brassica* from Sicily. It was noted that the Botanical Garden in Palermo will consider safety-duplicating its collections as well as hosting safety-duplicates from other genebanks.

Nordic Countries

Gert Poulsen presented a report prepared by Gry Synnevåg on the status of the collections maintained by the Nordic Gene Bank.

Poland

Iwona Bartkowiak-Broda informed the Group that the Polish Genebank at IHAR, Radzikow had recently been given the status of National Centre for Plant Genetic Resources. This change reflects the national coordinating role of this Centre and Poland's positive attitude towards long-term conservation of genetic resources.

The *Brassica napus* collection has been prepared for safety-duplication at CGN and HRI and will be transferred there as soon as agreements have been signed with these institutions. The data forwarded to the Bras-EDB now include extensive data of vegetable brassicas conserved at the Vegetable Research Institute in Skierniewice. A detailed report of these vegetable collections is included in this report (see Kotlinska, this volume).

Iwona Bartkowiak-Broda also informed the Group that, if given some financial support, IHAR could undertake to screen the Group's *Brassica napus* collections for glucosinulate content and fatty acid contents in order to provide standardized evaluation data to the Bras-EDB.

Portugal

João C. Silva Dias presented an update of the status of the Portuguese *Brassica* collections which are maintained at BPGV, ISA and HRI. He informed the Group that the collection maintained at ISA is basically considered an active collection and that no regeneration of the material maintained at BPGV has been done since the material was collected. He questioned the urgency of doing this since it is likely that about 50% of this material is duplicated in the collection at HRI. BPGV has conducted four collecting missions during the past two years, in Northwest Portugal and in the Inland Centre / North regions.

Spain

César Gómez-Campo reported that a national programme on plant genetic resources was established 2 years ago. This has promoted further collection of seeds and includes an agreement for systematic duplication of the samples in at least one of the MBG, CRF, UPV and SIA genebanks. Regeneration is considered a bottleneck for other activities and some centralized facilities for general use have been proposed.

The Netherlands

Ietje Boukema gave a status report of the Cruciferae collections maintained at CGN. The principal changes with regard to the report presented at the last meeting is the addition of 279 recently regenerated accessions of mainly *Brassica napus, B. oleracea* and *Raphanus sativus*. Passport data of the complete collection

are now available through Internet as downloadable ZIP files.[9] The Cruciferae collections maintained at CGN have been utilized intensively for breeding and research (1734 seed samples distributed during the past 2 years).

United Kingdom

Dave Astley informed the Group that a reference collection of 400 *Brassica napus* cultivars is maintained at the National Institute of Agricultural Botany, Cambridge. This collection which was not reported on at the previous meeting is well characterized and documented.

Safety-duplication

Thomas Gass introduced the subject by giving an overview of the past agreements reached at regional and global level to ensure the safety of *ex situ* collections. He emphasized that safety-duplication should be seen as an integral part of the overall efforts to ensure and rationalize the long-term conservation. In this regard he mentioned the discussions initiated for *Secale,* for which an attempt is being made to define a decentralized European collection made up of the total of national collections and within which each country would commit to maintaining its original material on behalf of the other countries.

In the discussion that followed it was felt that the idea of constructing a European *Brassica* collection needed more discussion and may currently be difficult to implement due to the lack of conclusions in the ongoing international negotiations about the International Undertaking. It was emphasized, however, that it is the responsibility of genebanks holding national base collections to ensure that these are safety-duplicated. The 'black box' arrangement whereby the genebank of origin has the responsibility for the quality of the stored material and its regeneration when required, was seen as currently the most cost-effective method of safety-duplication. The Group expressed its thanks to CGN and HRI for their continued commitment to maintain International *Brassica* Base collections since the 1980s.

It was agreed that genebank managers will check their collections and send to the Bras-EDB and the ECP/GR Coordinator by August 1997 data about the safety-duplication status and location of the safety-duplicates. The following genebanks offered to host safety-duplicates of *Brassica* collections within specific bilateral 'black box' arrangements:

- HRI
- CGN
- Palermo Botanical Garden
- the Nordic Gene Bank
- the Czech Gene Bank at RICP Prague-Ruzyne
- the Spanish Genebank is available to host a limited number of 'black box' safety-duplicates.

With regard to the large collections maintained at HRI and CGN the Group was informed that the safety-duplication was reciprocal between both institutes and that 75% of the collection at CGN and 50% of the HRI collection is already safety-duplicated. Since the last meeting, HRI has received no material for safety-duplication other than that from the Netherlands. CGN has received safety-

[9] URL: http://www.bib.wau.nl/cgn/

duplicates of 50% of the Belgian collection, as well as duplicates of the UK and Portuguese material included in the EU core collection project.

Studies on regeneration and rationalization methods

Brassica regeneration procedures at NGB

A report on the current regeneration procedures utilized at the Nordic Gene Bank was provided to each participant by Gry Synnevåg who was unable to attend the meeting. The Group expressed its thanks for the report (see Synnevåg, this volume).

Changes in gene/allele frequencies during regeneration

Mats Gustafsson presented the results of the project work on shifts in gene/allele frequencies following regeneration. He thanked the nine institutions which had collaborated by regenerating the two standard seed populations using their normal practices.

The results were presented for enzyme systems which exhibited polymorphism. He reported that considerable differences had been detected in the comparison of the original and regenerated material. But it was surprising that in some samples the variation had increased in comparison with the original populations. The number of plants analyzed per sample was high. The conclusion can be drawn that the method of regeneration, and probably also contamination, are the most likely explanations for this result.

For the *Brassica napus* accessions, wide variation was found both within and between the regenerated populations. For five of the populations, the differences were statistically significant in several of the enzyme systems.

In the diploid *Brassica rapa* accessions, it was possible to quantify the genetic variation within a population. The variation in allelic frequency allowed the differentiation between all nine populations. It was puzzling to find new alleles in some of the populations. Therefore each of the participating institutes should study their methods of regeneration practice, taking account of the results of this research which will be published shortly.

Rationalization of collections and related studies of the diversity spectrum

Studies on regeneration and rationalization of collections of kale, cabbage and cauliflower landraces in France

Grégoire Thomas presented preliminary results on a morphological and molecular study within and between landrace populations of kale, cabbage and cauliflower.

A pragmatic approach to the conservation of landraces was adopted through pooling populations. The resources were not available to regenerate large numbers of landraces of *Brassica oleracea* crops. Therefore, populations were combined, based on selection according to morphological and agronomic traits, and ecogeographic origin.

Studies were carried out in parallel on the effects of pooling on the genetic variability within the original and pooled populations. Experiments on pooling strategy and crossing systems covered the number of plants used per landrace, the density of plants in the pool and the design of the pool plants in the seed production.

Assessment of changes in variability are continuing with morphological, isozyme and RAPD markers. The work will continue with the production of secondary pools and subsequent marker analysis. The discussion was centered on the potential loss of genes and gene combinations from individual landraces compared to a pooled population.

Distribution of genetic diversity in Dutch white cabbage

Ietje Boukema presented the outline of a research project to evaluate the variability on Dutch white cabbage (see van Hintum *et al.* this volume). CGN rationalized the *Brassica oleracea* cultivar collections by bulking following extensive reviews of the material in field trials by breeders and other experts and by using isozyme markers. These isozyme studies still left unanswered the question whether one could go even further with bulking accessions.

The project will use AFLPs and microsatellites to clarify important questions for the management of genetic resource collections, particularly the distribution of the variation within the crop genepool. Aspects that will be considered are the collection size and the regeneration procedures necessary to maintain the variability. The results will provide guidance for managers of collections.

Rationalization of accessions of *Brassica oleracea* var. *ramosa* DC.

Klaus Dehmer presented the issue of the maintenance of vegetative kales which is costly and presents quarantine problems for other cruciferous crops maintained locally. The collection of Dr. A. Zeven has been transferred to CGN, with cuttings passed to IPK-Gatersleben. The collection was classified by Zeven into eight morphotypes on the basis of ploidy level and isozyme types.

Sixty-two clones were examined by RAPD analysis at IPK-Gatersleben and assigned to seven groups designated A to G. By combining all these data, it is possible to allocate clones into eight classes and identify putative duplicate clones. Probably, morphotype variation is influenced by viral infection and further work will promote the identification of duplicates. Attempts will be made to transfer the clones to *in vitro* culture in order to eradicate viruses and provide ongoing maintenance.

Tests on germination rates of *Brassica* seeds showed that for a storage temperature of -15°C with an average humidity of 4-6%, no major problems in loss of viability were encountered for periods up to 20 years.

Vernalization procedures for seedlings

Some *Brassica rapa* and *B. napus* accessions can be induced to flower by vernalization during the seedling stage due to the 'absence' of a juvenile phase.

João C. Silva Dias presented the results of a small research study made by his group where they studied the effect of seedling vernalization at 4°C for 2, 4, 6 and 8 weeks on six genotypes of *Brassica rapa* (one Chinese cabbage and five turnips), three of *B. napus* (two vegetable rapes and one rutabaga) and nine of *B. oleracea* (one annual Kailan, four Tronchuda cabbages, one white cabbage, one Savoy cabbage, one Brussels sprouts, one broccoli). The study confirmed that seedling vernalization is effective in *B. rapa* and in the two vegetable rapes (*B. napus*). It was not effective for a majority of the *B. oleracea* and for rutabaga. These facts should be taken into consideration for the regeneration of accessions of these species.

The quality of containers for base collection storage

César Gómez-Campo demonstrated a range of containers used by different genebanks. He has tested the hermetic quality of these materials under high humidity conditions and presented his conclusions.

He stressed the importance for each collection manager to maximize the longevity of their seed accessions by ensuring the quality of packaging material for long-term storage.

The question of plasticizers from plastic containers having detrimental effects on seed variability was discussed. The Group agreed that a curator must consider this problem as a part of the evaluation of packaging materials. Gómez-Campo offered to test any container of prospective interest of the Working Group members, using the same standardized conditions as those in his project.

Recommendations

The Group recognized the need for ways of rationalizing the collections and improving the regeneration procedures.

The meeting appreciated the wide range of research activities carried out in this direction within the Group and is looking for further guidelines that should emerge from the results expected in the next few years. In particular the Group supports the research of CGN and G. Thomas on bulking similar accessions and subsequent investigations in changes of variability using molecular markers. It was agreed that further research along these lines should be done, taking account of the breeding systems and evaluating agronomic modifications of the bulked populations.

Genebank managers were reminded of the importance to choose the most reliable containers (e.g. sealed glass vials) for their accessions, in order to minimize the need for regeneration.

The Group acknowledged the opportunity offered at the ISHS meeting in Rennes (September 1997) to present results on regeneration research.

Grégoire Thomas and Mats Gustafsson offered to peer review the publication: *'Regeneration of accessions in seed collections: a decision guide'*, under preparation by N.R. Sackville Hamilton and K.H. Chorlton (IGER, UK), keeping in mind the specific problems of the regeneration of *Brassica* and therefore ensuring that this guide covers in a satisfactory way the needs of a *Brassica* germplasm manager. The decision guide will be distributed to all participants as soon as possible following publication.

Core Collection development for *Brassica*

Dave Astley outlined the development of a 'core collection' for use in the EU AIR *Brassica* project as described in the report of the Second Meeting in Lisbon. He went on to describe the conclusions which have been drawn from the use and development of the work (see Astley, this volume).

The value of the *Brassica* EDB as a tool in developing the collection was essential. The application of a simple analytical tool, the path indicator, to structure the genepool represented in the *Brassica* EDB in a hierarchical form provided significant momentum to the project.

The conclusion of the EU project is that the availability of these tools provides high flexibility to meet demands of users of 'core collections', according to defined

criteria. New 'core collections' can be developed after these criteria have been agreed upon by the users, the central crop database manager and the curators.[10]

The Group recognized that such an approach could increase the workload of the central crop database manager and that new 'core collections' will only be developed to meet the needs of projects with more than three participants from ECP/GR countries.

In situ conservation

In situ conservation of *Brassica oleracea* wild relatives

A group of at least nine wild species of *Brassica*, related to cultivated brassicas, is distributed along the Mediterranean and Atlantic coasts from Israel and Cyprus to Wales. During a series of missions, supported by IPGRI and with the aim of collecting germplasm, over 200 population sites have been visited. Actual or potential threats have been observed in at least half of the sites. Overgrazing, nearby constructions, fires, quarrying, competition by alien species and nitrification of the cliffs are among the most conspicuous threats.

The prospects for *in situ* conservation of the wild *Brassica* species were discussed at the second ECP/GR *Brassica* Group meeting in Lisbon, Portugal in 1994. It was recognized by the Group that the *ex situ* conservation of *Brassica* was well advanced and that it was the proper time to consider the ways in which *in situ* conservation could complement these activities. The Group also recognized Sicily, with its populations of the species *Brassica macrocarpa*, *B. villosa* and *B. rupestris*, to be a suitable target area to gain knowledge about the population genetics and to develop *in situ* strategies. In 1994 it was also agreed that César Gómez-Campo and Mats Gustafsson would collaborate with Francesco Raimondo (Palermo) and IPGRI to further develop the strategy on *in situ* conservation of the above-mentioned taxa.

During the 2-year period separating the two meetings (1994-96) the following actions have been taken:

Monitoring of certain 'key' populations

Lorenzo Maggioni reported on the monitoring he carried out on populations of *Brassica rupestris* and *B. villosa* from Sicily, *B. macrocarpa* from the Egadi Islands and *B. incana* from Sicily and the Tremiti Islands (see Maggioni and Eastwood, this volume). He also reported on a similar activity carried out by Antonia Eastwood on *B. incana* populations in Croatia. The results of these investigations identified sites which were previously unknown, apparent fluctuations in population size and structure with time, and even the disappearance of other populations. The existence of known threats were confirmed. Maggioni concluded that there is an urgent need for more effective legislation for the protection and conservation of wild *Brassica* populations.

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[10] See following references:

Boukema, W.B., Th.J.L. van Hintum and D. Astley. 1997. The creation and composition of the *Brassica oleracea* Core Collection. Plant Genetic Resources Newsletter 111 (in press)

Ellis, P.R., D.A.C.Pink, K. Phelps, P.L Jukes, S. E. Breeds and A.E. Pinnegar. Evaluation of a core collection of *Brassica oleracea* accessions for resistance to *Brevicoryne brassicae*, the cabbage aphid. Euphytica (in press).

Recent activities and planning for *in situ* conservation in Sicily

On behalf of Francesco Raimondo, Giuseppe Venturella reported the actions taken to initiate *in situ* conservation. It was recommended by Venturella that the first step was to target populations located inside existing natural reserves. The following sites were suggested:

- the island of Marettimo, to protect populations of *Brassica macrocarpa*
- the Zingaro reserve in the Trapani district to protect populations of *B. villosa* subsp. *drepanensis*
- the Madonie park in the Palermo district to protect populations of *B. rupestris*
- the Pellegrino mountain in Palermo district to protect populations of *B. rupestris*
- the Nebrodi natural park in the Messina district to protect populations of *B. incana*.

In the near future, the following actions will be taken by the Palermo Institute:

- to contact the local administration and to discuss the possibility of increasing the grazing along the foothills in order to reduce the vegetative production and eliminate fires
- to plan the monitoring of the demographic structure of the populations
- to stimulate further characterization of the infraspecific taxa, e.g. by applying molecular marker systems and seed physiology.

Inclusion of wild *Brassica* species in lists of endangered plant species

At the 1994 *Brassica* Working Group meeting, it was suggested to investigate the possibility of including the Sicilian *Brassica* species on lists of protected plants such as the Bern Convention and the European Red List. César Gómez-Campo reported that his inquiries to the Spanish representative in the Council of Europe resulted in the recommendation that Italian scientists should get in contact with the Italian Ministry of Environement and discuss the possible inclusion of the Sicilian endemic *Brassica* species in the Bern Convention list.

Recommendations

The Group agreed that the conservation of the endangered populations of Sicilian ($2n$=18) wild brassicas should be pursued vigorously. At the same time it was acknowledged that any effort in this direction should start under the coordination of an Italian institution. This would be the most appropriate way to approach both Italian and European authorities in the attempt to seek for legislative formulae that could facilitate the conservation of wild brassicas. The Group recognized F. Raimondo as the most suitable scientific authority to be the promoter of activities in this direction and to contact the appropriate Italian authorities when needed. Parallel actions taken by P. Perrino would be considered valuable.

The initiative of F. Raimondo to propose management plans for the existing Sicilian nature reserves, in order to ensure the *in situ* conservation of *B. macrocarpa, B. rupestris* and *B. villosa*, including the infraspecific taxa *drepanensis* and *bivoniana*, was welcomed and backed by the Group.

Management actions intended to prevent the occurrence of fires threatening the populations were considered very important. At the same time the institution of a regular monitoring activity of the size and structure of the populations was considered a primary activity that the Group would suggest to include in the management of the reserves. The extension of the monitoring activity to other populations of *B. macrocarpa, B. villosa* and *B. rupestris* living outside currently protected areas, was considered an activity to be encouraged.

Mats Gustafsson and César Gómez-Campo offered their availability for collaboration and advice in the definition of the management plan proposals to the Italian authorities. They wish to cooperate with F. Raimondo for the formulation of projects for the *in situ* conservation of the wild brassicas in those areas where they are currently under threat.

The inclusion of *B. villosa* and *B. rupestris* in the list of protected plants of the Bern Convention was still seen as a useful step to be taken to help the conservation of these wild populations. It was agreed that appropriate actions in this sense from F. Raimondo and independently from P. Perrino would increase the chance to reach the objective. Pietro Perrino explained that there will be opportunities to take the necessary contacts with the Italian and European authorities through different channels (e.g. C.N.R. and the Ministry of Environment) and he was encouraged to do so.

The Group suggested that, depending on the availability and organizational support of F. Raimondo at the Botanical Garden of Palermo, a small workshop could be organized in Palermo where both European wild *Brassica* and conservation experts could meet with Italian authorities. In this context the status and importance of the Sicilian wild brassicas could be illustrated and the formulation of a feasible plan of action for their better conservation could be speeded up.[11]

Project resubmission to the EU genetic resources programme (EC1467/94)

Ietje Boukema reported on the outcomes of the submission of the *Brassica* Plant Genetic Resources project to the first call for proposals of the EU genetic resources programme in 1995. As a result of this first call for proposals only eight projects were approved; the *Brassica* project scored a grade B evaluation and was not included among the projects selected for funding. The project was not resubmitted to the second call for proposals. The Group was asked to express its opinion on a resubmission to a possible third call in 1997 or 1998.

Comments received from the evaluation board, explaining the points of criticism, were explained to the Group, in order to use them as possible guidelines for the improvement of the project. The critical comments are the following:

- possible duplication of some of the activities with those of the ongoing AIR Project
- lack of clarity in identifying clients or end users of the project
- absence of application of standard molecular techniques
- presence of more than one partner per country

[11] A workshop on wild Sicilian brassicas was held in Palermo on 19 April 1997. As an outcome of the meeting, the necessary steps for the inclusion of these wild brassicas in the Bern Convention list of protected plants were initiated. A project for *in situ* conservation in Sicily will also be submitted to the Italian authorities.

- absence of private partners in the project.

The project group considered some of this criticism as unjustified, since the project did accurately follow the guidelines for applications. The perspectives for a possible successful resubmission of the project were discussed. Concern was expressed for the uncertainties of both the availability of funds in the next year from the EU for the EC 1467/94 programme and the frequent inconsistencies that were noted in the criteria used to evaluate the project proposals.

The amount of work and commitment necessary for the preparation of any project submission was highlighted as a critical aspect to compare with the expected chances to obtain limited EU funding.

On the basis of these discussions, the meeting agreed that, with a limited effort for improving the project along the lines of the criticism received, it should still be resubmitted if the possibility is given.[12] In order to do this, the following rearrangements to the project were agreed by the Group:

- reduction of the number of partners to one per country, where each partner will have the opportunity to split his tasks with subcontracting collaborators
- inclusion of one or more private breeding companies as partner(s) in the project to carry out specific evaluation and characterization
- inclusion of complementary molecular work for genetic diversity characterization already planned or in progress by the partner institutes. No funds will be requested for implementation of this ongoing research.

ISHS Symposium in Rennes

Grégoire Thomas announced that the next ISHS Cruciferae Symposium will be held in Rennes, France, 24-27 September 1997. During the meeting a special session will focus on plant genetic resources where breeders, plant genetic resources researchers and curators will have the opportunity to present their points of view. Representatives of the ECP/GR *Brassica* Working Group will be invited to give two presentations of 15 minutes each, as an opportunity to broadly outline the work done by the Group and to show one specific example of its activity. G. Thomas suggested that the ECP/GR *Brassica* Working Group take the opportunity to hold a one-day *ad hoc* meeting just before the ISHS symposium.

The Group welcomed and accepted this invitation. Thomas Gass indicated, however, that only a partial financial support for the participation of the Working Group members could be expected from ECP/GR. He also recommended that this extraordinary meeting be seen as a last opportunity, during Phase V, to give high visibility to the Working Group's achievements. This would be especially important in view of the evaluation of the *Brassica* Working Group's activities as part of the meeting of the ECP/GR Steering Committee, in 1998.

The meeting agreed that an extraordinary session of the *Brassica* Working Group would be held on 23 September 1997 in Rennes. In response to G. Thomas' invitation, the Group decided that L. Maggioni will present, during the ISHS meeting, the activities of the *Brassica* Working Group, while I. Boukema will offer an on-line presentation of the European *Brassica* Database.

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[12] By the time of going to press, it is known that the EC will not offer a new call for proposals in 1997, but the opportunity will be given in 1998.

The meeting suggested that F. Raimondo could offer to give a presentation on the status of *in situ* conservation of wild brassicas in Sicily and recommended the ISHS Scientific Committee to include this contribution in the genetic resources session.

Newsletter

Grégoire Thomas announced that the Cruciferae Newsletter can offer a 'page' for the ECP/GR *Brassica* Working Group. This would enable the Group to print useful information and give information about its activity to the Cruciferae community. The Group agreed on the value of this solution and agreed to take advantage of this already existing newsletter. A summary of the present meeting will be sent to G. Thomas before 15 December 1996 for inclusion in the next issue of the Cruciferae Newsletter.

Concluding remarks

During the final session of the meeting, the 'Discussions and Recommendations' were reviewed and adopted by the participants after a number of modifications.

Grégoire Thomas was elected new Chair of the ECP/GR *Brassica* Working Group. He will take up this position after the ISHS Symposium in Rennes (September 1997). Mats Gustafsson will remain in charge of the Group until then.

The Group expressed its thanks to the organizers of the meeting.

Presented papers

Report of the Chairperson on the period 1994-1996

Mats Gustafsson
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I welcome you all to this third meeting of the ECP/GR Working Group and especially those of you which participate for the first time. I hope that we will have open-minded discussions during this meeting and that the outcome will be fruitful.

My report covers the period from December 1994 to November 1996. A workplan was initiated and agreed upon at the second meeting in Lisbon. I will spend a couple of minutes to comment on some of the actions and activities which have been performed during the 2-year period since then.

European *Brassica* database

In November 1994, the Bras-EDB database, managed by Ietje Boukema at CGN, included 11 958 accessions originating from 15 countries and the proportion of the different species was 57% for *Brassica oleracea*, 17% for *B. napus* and 15% for *B. rapa*. According to I. Boukema, the database has increased in size during this period and now includes 12 849 accessions with about the same proportion between the three most important crop species – *B. oleracea*, *B. napus* and *B. rapa*. Some of the missing data in 1994 have been provided to CGN, but some information is still lacking and I. Boukema will report on the current status. In addition, the available information in the Wild *Brassica* database has been included in the Bras-EDB.

Duplicates

CGN will continue to try to identify duplicates. So far, over 1500 presumed duplicates have been traced.

Regeneration practices

Regeneration of the material stored in genebanks is a common problem. The brassicas are more or less outbreeding species and most of the stored material is heterozygous and heterogeneous. Thus, regeneration procedures should ensure that crossing between accessions is avoided, outbreeding within an accession is favoured as far as possible, the proportion of selfing minimized, and that the pressure to favour any particular genotype is small. A working group consisting of Gry Synnevåg, Yves Hervé and João Silva Dias, was formed at the 1994 meeting with the option to discuss and develop a protocol for regeneration procedures. The discussion is not yet concluded, but G. Synnevåg will report on practices used at the Nordic Gene Bank, especially concerning horticultural crop plants of *Brassica*. In addition, it has been concluded from the results of the isozyme studies (see below) that it would be desirable to develop a standard regeneration procedure that ensures the maintenance of the genetic variability of the original accession.

Effects of regeneration on gene and allele frequencies

The aims of the project were to assess genetic changes in a number of isozyme systems in two populations of annual *Brassica* species, *B. rapa* and *B. napus*, which have been regenerated at various geographical sites throughout Europe. The material, the regeneration procedure, the methods used for analysis of isozyme patterns and the results will be presented at this meeting. However, it is worthwhile to point out that the genetic variability of some of the populations has decreased while others have increased their genetic variability during the regeneration procedures. Comparisons made between regeneration sites show that considerable differences in allele frequencies occur.

In situ conservation

In 1994, César Gómez-Campo and Mats Gustafsson presented prospects and strategies to conserve wild *Brassica* species *in situ*. They pointed out that although many *Brassica* populations are located at sites difficult to access, others could be considered threatened. The working group recognized the suitability of Sicily with its populations of *B. macrocarpa*, *B. villosa* and *B. rupestris* as first foci to gain knowledge about the population genetics and to develop *in situ* strategies. It was also agreed that a collaboration with Francesco Raimondo (Palermo Botanical Garden) and IPGRI would be desirable to further develop the strategy on *in situ* conservation of these taxa. Since the 1994 meeting different actions have been taken, e.g. monitoring of certain key populations (Lorenzo Maggioni and Antonia Eastwood). Francesco Raimondo has agreed to promote these actions and to initiate *in situ* conservation of some key populations, and under the supervision of Mats Gustafsson, a PhD student (C. Lannér) studied genetic diversity in various populations of the Sicilian taxa.[13] The results of the initiatives of the Palermo Botanical Garden in Sicily will be presented by Giuseppe Venturella.

Inclusion of wild *Brassica* species in lists of endangered species

Contacts have been made with country representatives in the Council of Europe to obtain information about the procedures for the modification of the Red List of endangered species. It was stated that the ECP/GR group may support an inclusion of endemic *Brassica* species in the lists, but the formal application must be made by Italian authorities. César Gómez-Campo will report on the results of these inquiries and initiate a discussion about further actions in this respect.

Brassica core collection

The development of a *Brassica* core collection was discussed and agreed upon at the second meeting. The original intention included the participation of different genebanks for the development of a general European *Brassica* core collection, with CGN as a coordinator for *B. oleracea*, HRI for *B. rapa* and NGB for *B. napus*. However, no further actions have been taken during the last 2 years owing to lack of financial support.

Project submission to the EU genetic resources programme

In 1995, a proposal of financial support for the *Brassica* Plant Genetic Resources project was submitted to the EU programme. The *Brassica* project obtained a

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[13] Lannér, C. 1997. Genetic relationships within the *Brassica oleracea* cytodeme. Comparison of molecular marker systems. PhD Thesis, Swedish University of Agricultural Sciences, Svalöv. Acta Universitatis Agriculturae Sveciae. Agraria 39.

grade B evaluation and was not included among the eight projects selected for funding. The prospects for a possible resubmission of the project will be discussed during this meeting.

Brassica Newsletter

It was recommended at the Lisbon meeting to try and start a *Brassica* newsletter. So far however, no newsletter has been prepared because of a lack of contributions.

Thank you for your attention and let us proceed to the next point!

The European *Brassica* Database

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Introduction

The European Database for *Brassica* (Bras-EDB), developed by the CGN, was presented at the ECP/GR *Brassica* meeting, 13-15 November 1994 in Lisbon, Portugal (Boukema *et al.* 1995). The database focuses on passport data of the genus *Brassica* maintained in germplasm collections in European research institutes and genebanks.

After the meeting in 1994 in Lisbon the Bras-EDB was expanded with data from collections which had not been included yet. The database now includes, besides cultivated material, wild *Brassica* species. The Bras-EDB and the data-dictionary, describing the fields in the Bras-EDB, are available on request in several formats.

Content of the Bras-EDB

Currently (November 1996) the database contains 12 849 accessions from 21 collections in 17 countries (Table 1). The database holds a wide variety of *Brassica* crops, e.g. vegetables, oil and fodder crops and wild species. *Brassica oleracea* is the largest species in the Bras-EDB (56%), followed by *B. napus* (17%) and *B. rapa* (16%). The major part of the accessions of which the population type is known are cultivars (63%, corresponding to 38% of the total), but landraces and wild species also are included. The major taxonomic groups per collection are given in Table 1 and the population types per collection in Table 2.

Duplicates

The identification of duplicates will help genebanks to set priorities for regeneration. Duplicates are traced by matching names or parts of names with a similar sound, as explained in the 1994 meeting (Boukema *et al.* 1995) or by matching collection or other numbers. The work on tracing duplicates, which is very time-consuming, is an ongoing activity.

Utilization

The Bras-EDB was requested by several genebanks, research institutes and private breeding firms in and outside Europe, but its actual use is difficult to assess. The database was used intensively for the creation of a *Brassica oleracea* core collection (BOCC) for the EU project entitled "The location and exploitation of genes for pest and disease resistance in European genebank collections of horticultural brassicas" (Leckie *et al.* 1996). The accessions belonging to this core collection are marked in the Bras-EDB. A new tool, the BOCC path indicator, used for structuring the core collection, also is included in the Bras-EDB (Boukema *et al.* 1997).

Table 1. Number of accessions in the Bras-EDB per collection and major taxonomical group

Data source	*B. carinata*	*B. juncea*	*B. napus*	*B. nigra*	*B. oleracea**	*B. rapa*	*X Brassi-coraphan.*	Wild species	Others & unknown	Total
Belgium	–	–	48	–	22	93	–	–	–	163
Bulgaria	–	37	271	24	153	75	–	–	8	568
Czech Republic	1	37	502	8	231	68	–	–	29	876
France	–	–	80	–	524	32	–	–	–	636
Germany (Braunschweig)	115	93	260	95	390	195	–	1	1	1150
Germany (Gatersleben)	37	75	118	38	652	295	1	35	181	1432
Greece	–	–	–	–	125	–	–	43	1	169
Hungary	–	–	–	–	95	4	–	–	–	99
Italy	–	6	13	2	242	166	–	9	71	509
the Netherlands	108	22	90	24	526	335	11	2	12	1130
Nordic Countries	–	–	94	1	167	83	–	–	–	345
Poland	–	–	301	–	101	7	–	–	–	409
Portugal (Lisbon)	–	–	–	–	51	–	–	–	–	51
Russia	21	–	–	–	1083	112	–	–	–	1261
Spain (Galicia)	–	–	36	–	178	144	–	–	4	362
Spain (Madrid, CRF)	–	–	1	–	42	17	–	–	–	60
Spain (Madrid, UPM)	–	–	–	–	60	–	–	179	–	239
Spain (Valencia)	–	–	27	–	153	–	–	–	38	218
Switzerland	–	–	5	–	99	18	–	–	–	122
Turkey	–	–	15	65	137	21	–	–	2	240
United Kingdom	8	45	301	1	2122	359	–	5	14	2855
Total	**290**	**315**	**2162**	**258**	**7153**	**2024**	**12**	**274**	**361**	**12849**

* Including wild *B. oleracea* accessions.

Table 2. Number of accessions in the Bras-EDB per collection and population type

Data source	Wild	Weedy	Land-race	Cultivar	Research material	Un-known	Total
Belgium	–	–	70	75	16	2	163
Bulgaria	–	–	–	127	4	437	568
Czech Republic	–	–	23	716	135	2	876
France	–	–	636	–	–	–	636
Germany (Braunschweig)	12	–	6	586	18	528	1150
Germany (Gatersleben)	35	–	–	–	–	1397	1432
Greece	44	18	–	16	91	–	169
Hungary	–	–	50	44	5	–	99
Italy	9	–	–	–	–	500	509
the Netherlands	8	–	248	723	100	51	1130
Nordic Countries	–	–	–	–	–	345	345
Poland	–	–	–	–	–	409	409
Portugal (Lisbon)	–	–	51	–	–	–	51
Russia	–	–	–	–	–	1216	1216
Spain (Galicia)	–	–	336	26	–	–	362
Spain (Madrid, CRF)	–	–	60	–	–	–	60
Spain (Madrid, UPM)	235	–	–	–	–	4	239
Spain (Valencia)	–	–	121	64	–	33	218
Switzerland	–	–	–	122	–	–	122
Turkey	–	–	97	–	–	143	240
United Kingdom	21	–	346	2406	15	67	2855
Total	**364**	**18**	**2044**	**4905**	**384**	**5134**	**12849**

Future activities

The following activities are planned for the next 4 years, provided that the necessary capacity will be available:

- Inclusion of data other than passport data, such as characterization data according to the minimum descriptors (Gass *et al.* 1995).
- Updating of all data sets in the Bras-EDB, resulting in a complete new edition.
- Inclusion of the Bras-EDB in the system of the central crop databases under the ECP/GR homepage on the Internet.
- Further analyses of the data in the Bras-EDB, resulting in identification of 'gaps' in the combined collections. These gaps can be related to other, non-European *Brassica* collections, such as the USDA collection.

It is hoped that the Bras-EDB will become the central information source used for the coordination of activities on the conservation of *Brassica* germplasm in Europe.

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Status of *Brassica* genetic resources collections

Status of the Bulgarian national collection of crucifers

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The national collection of crucifers in Bulgaria consists of 998 accessions of *Brassica* and 156 of *Raphanus, Sinapis, Camelina, Crambe* and *Eruca.* These include 37 local forms and 256 breeding lines. From 1978 to 1996, 1045 cultivars and lines were characterized. The IIPGR in Sadovo maintains 205 accessions from *Brassica oleracea* subsp. *capitata* (Table 1). The *Brassica napus* collection consists of 271 accessions, *B. rapa* 75, *B. juncea* 37 and *B. nigra* 24. Other cruciferous crops are represented by 156 accessions. Most of the *Brassica* accessions have been characterized according to international descriptors. The collection was evaluated by Neykov (1983, 1989) and Angelov (1990).

In the IIPGR-Sadovo 622 accessions are stored in long-term storage at –18°C. They include 497 accessions of *Brassica (B. oleracea* 157, *B. napus* 259, *B. rapa* 34, *B. juncea* 30, *B. nigra* 17) and 125 accessions of others. For the pollination of cultivars in the long- and short-term storage, beehives located outside the isolation cages, microfamilies of bees inside the cages and flies were used (Neykov 1996). These methods are more effective and lower cost than traditional manual methods. The requirement for long-term conservation is to keep a minimum of 5000 seeds per accession. Short-term preserved seeds are used as the initial material for improvement through breeding at the institute and for international exchanges.

Several multicrop collecting missions were performed in the country: Rodopa and Belasitsa mountains, Black Sea coast, Northeast, Central Southeast Bulgaria. Wild species of *Brassica rapa, B. nigra, B. juncea, Sinapis alba, Sinapis arvensis, Camelina sativa, Crambe maritima, Conringia orientalis* and local forms of *Brassica capitata* were collected during these missions.

The Institute of Vegetable Crops-Maritsa, Plovdiv, maintains 96 *Brassica* accessions (cultivars and breeding material) of which 75 are *B. oleracea* subsp. *capitata* and 21 are other vegetable species.

The ESVC-Gorna Oryahovitsa maintains 150 cultivars and 150 breeding lines of *B. oleracea* subsp. *capitata botrytis* and others.

The CES-Sandanski maintains 60 breeding lines of *B. oleracea* subsp. *capitata*.

Workplan

Collecting missions, evaluation and regeneration of the *Brassica* accessions will continue. Breeding activities will continue to include the following traits: disease resistance, winter and winter-spring cultivars, earlier forms, winter storage, chemical structure (dry matter, vitamin C, sugar, oil, protein).

Table 1. The Bulgarian collections of *Brassica* and other cruciferous crops

Species	Subsp./variety	Collected passport data	Landraces	Breeders material	Characterized	Stored	Regenerated
IIPGR – Sadovo							
Brassica oleracea	*capitata*	205	18	15	185	157	190
	botrytis	24	–	–	24	–	–
	gemmifera	14	–	4	12	–	6
	sabauda	9	–	–	9	–	6
B. napus	*oleifera*	271	–		259	259	259
B. napus	*rapifera*	18	2	–	16	–	5
B. rapa	*oleifera*	75	4	–	31	31	51
	pekinensis	5	–	2	5	3	5
B. juncea		37	2	–	30	30	50
B. nigra		24	3	–	17	17	24
Camelina sativa		51	2	–	51	51	53
Crambe abyssinica		18	2	–	18	18	20
Eruca sativa		3	–	–	3	3	3
Raphanus sativus	*radicula*	35	–	–	30	4	10
Sinapis alba		49	4	–	40	49	53
IVC – Maritsa							
B. oleracea	*capitata*	50	–	25	75	–	75
	botrytis and others	21	–	–	–	–	15
ESVC – G. Oryahovitsa							
B. oleracea	*capitata*	100	–	150	130		150
	botrytis and others	50	–	–	50	–	30
CES – Sandanski							
B. oleracea	*capitata*	60	–	60	60	–	40
Total		**1119**	**37**	**256**	**1045**	**622**	**1045**

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Status of the national *Brassica c*ollection in Croatia

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Introduction

On the territory of the Republic of Croatia, as a part of the Mediterranean genepool, the following wild species of brassicas can be found: *Brassica nigra, Brassica oleracea, Brassica campestris, Brassica napus* as well as the following endemic species of the same genus:

- *Brassica botterii* Vis. syn. *Brassica incana* Ten. subvar. *botterii* (Vis.) Hayek on the isle of Palagruza
- *Brassica mollis* Vis. on the isle of Korcula
- *Brassica cazzae* Ginzberger and Teyber.

Furthermore, local conditions allow the successful cultivation of hybrids, modern cultivars and primitive varieties of cabbage, Savoy cabbage, Brussels sprout, broccoli, cauliflower, kohlrabi, chinese cabbage and collard.

The national *Brassica* collection

In the last few decades there were some attempts at collecting and evaluating brassicas (Pavlek 1955; Mikolcevic 1960), but systematic work started in 1987 with the establishment of the Yugoslav Plant Gene Bank. The collection consists of:

a) 15 accessions of collards
b) 11 accessions of intermediate between collard-cabbage and Savoy
c) 2 accessions of cabbages
d) 1 accession of broccoli.

These samples were collected from the surroundings of Dubrovnik (Brgat, Konavli) (a), Zadar (b), Nedeljanec (c), Župa Dubrovacka (d), the Mostar region (Bosnia and Herzegovina) and the isles of Hvar (V. Grablje), Korcula (Pupnat, Zrnovo, Blato), Brac (Bol), Vis (Komiza) and Mljet (Polaca).

As the genus has a biennial and open-pollinated character, and money and space were lacking for regeneration of all the populations in the institutions which collected and evaluated them, it was decided to regenerate the accessions on farm. However, war prevented collecting in the region of origin of the accessions and in the neighbouring areas (isles).

The collection now consists of samples that were collected at the end of the 1980s, kept in inadequate storage conditions. Most have lost their viability. Parts of the collection have been characterized and/or evaluated according to IPGRI (IBPGR) descriptor lists, slightly modified.

A computerized *Brassica* database is not operational yet.

Research activities

It is high time to start collecting local populations again because of their considerable stress resistance. These populations are grown only for self-consumption, mostly in old people's households. On the market-oriented farms introduced varieties and hybrids of well-known European seed companies predominate.

The activities of the Croatian Bank of Plant Genes are starting but organizational and financial problems make it impossible to launch an expedition

in order to collect accessions. Therefore friends and colleagues are requested to send seeds of old varieties and autochthonous populations. It was decided to concentrate the activity of the Croatian Bank of Plant Genes on landraces of cabbages, cauliflower, broccoli and especially collards.

Accessions of cabbages, broccoli and collards are evaluated by the Vegetable Center Zagreb, while the Institute for Adriatic Crops in Split describes the populations of autochthonous winter cauliflowers.

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Status of the Czech *Brassica* collection

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Since the last meeting of the *Brassica* Working Group some changes have occurred in the status of the Czech *Brassica* collections. There are two main institutions maintaining collections of *Brassica* genetic resources in the Czech Republic. The station of the RICP Prague-Ruzyne Gene Bank, in the former Research Institute for Vegetable Breeding and Growing in Olomouc, maintains the collection of predominantly vegetable crops. This collection had to be revised recently because of a great number of limited or no longer available accessions, due to the loss of viability of seeds. Also some breeders' materials obtained by inbreeding of F_1 hybrids were excluded. Accessions with a limited availability and a small amount of seeds have been prioritized for the conservation efforts according to type and quality.

A collecting mission to Slovakia in 1995 brought ten landraces of cabbage, which have been included into regeneration procedures. Some populations of a northeastern Bohemian landrace of pointed headed red cabbage have been collected as well. The collection also has been supplemented with 18 accessions obtained from VIR (St. Petersburg, Russia), including five old Czech landraces of cabbage that had been lost formerly.

The collection of oil crops in the Research Institute of Oilseed Crops (RIOC) in Opava has not shown any changes.

A new small collection of fodder *Brassica* crops, especially fodder kales, is being established in the Research Institute for Fodder Crops (RIFC) in Troubsko near Brno.

According to the workplan of the 2nd *Brassica* Working Group meeting, revised data of the Bras-EDB from the Czech Republic were provided to the Bras-EDB centre in Wageningen at the beginning of 1996.

The Czech collections were revised both in the RIOC, Opava and the RICP station at Olomouc and the conservation efforts have been prioritized. Both Czech collections, which include *Brassica* spp. in RIOC, Opava and in the RICP Genebank station, Olomouc, are intensively regenerated, depending on working capacities and technical facilities. Regenerated seed samples are maintained in long-term seed store of the Genebank in RICP, Prague-Ruzyne. Fast regeneration of all collections is considered as the top priority of the National Programme on Plant Genetic Resources.

Tables 1 and 2 show a review of the current status of the *Brassica* collections in the Czech Republic.

Table 1. *Brassica* L. in the collections of RICP Olomouc and *RIFC Troubsko (vegetables and fodder crops)

Species	Subsp./variety	No. accessions in collection	Status			No. accessions stored in the GB
			Landraces	Advanced cvs.	Breeders mat.	
B. oleracea L.	*acephala* *	10*	–	10*	–	10*
	botrytis	54	–	53	1	13
	capitata	70	25	43	2	14
	gemmifera	5	–	5	–	3
	gongylodes	26	–	20	6	9
	italica	1	–	1	–	1
	sabauda	8	–	8	–	7
	sabellica	5	–	5	–	–
B. rapa L.	*pekinensis*	16	10	6	–	3
	rapa	1	–	1	–	1
Total		**196**	**35**	**152**	**9**	**61**

Table 2. *Brassica* L. in the collection of RIOC Opava

Species	Subsp./variety	No. accessions in collection	Status			No. accessions stored in the GB
			Landraces	Advanced cvs.	Breeders mat.	
B. napus L.	*oleifera* (winter)	435	–	315	120	150
	oleifera (spring)	131	1	119	11	41
	rapifera	2	–	2	–	–
B. rapa L.	*oleifera (biennis)*	31	2	28	1	4
	oleifera (praecox)	22	–	18	4	11
B. nigra (L.) Koch		11	–	8	3	10
B. juncea (L.) Czern.		40	–	29	11	12
Total		**672**	**3**	**519**	**150**	**228**

Status report on the B*rassica* collections in German genebanks

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With contributions from L. Frese, BAZ; U. Freytag, T. Gladis, H. Knüpffer, H. Lux, C.-E. Specht, E. Willner, IPK; and A. Zeven, Dept. of Plant Breeding, Agricultural University Wageningen, The Netherlands.

Of the 41 species belonging to the genus *Brassica*, 27 are maintained at the IPK Genebank in Gatersleben, 6 at the BAZ Genebank in Braunschweig, and 13 species at the External Branch Station of the IPK Genebank in Malchow/Poel (taxonomical classification according to Gladis and Hammer 1990). In recent collecting missions from Gatersleben (Table 1), a total of 28 *Brassica* accessions was sampled, while 140 accessions were obtained from several institutions or persons.

The total of accessions stored at the BAZ/Braunschweig (1212), at the IPK/Gatersleben (2043) and at the IPK/Malchow (1453) amounts to 4708 accessions (Table 2). More than 90% of these accessions (4349) belong to the six economically important *Brassica* species, i.e. *B. carinata*, *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea* and *B. rapa* (Tables 2, 3; for the region of origin see Table 3), with the largest collections of the respective species being conserved at BAZ/Braunschweig (*B. carinata*, *B. nigra*), IPK/Gatersleben (*B. juncea*, *B. oleracea*, *B. rapa*) and IPK/Malchow (*B. napus*).

Besides the maintenance and multiplication of the collection, tests on the germination rates of *Brassica* seeds were conducted at Gatersleben. In general, no major problems were encountered with the *Brassica* species within periods up to 20 years (Figs. 1, 2) when storing the material at a temperature of –15°C and an average humidity between 4 and 6%.

In an attempt to reduce the time and work needed to maintain vegetatively propagated *Brassica* species, morphological, cytological and isozyme analyses (CPRO-DLO Wageningen) and PCR-based analyses (IPK Gatersleben) were conducted on accessions of perennial kale (*Brassica oleracea* L. var. *ramosa* DC.) from the Netherlands (57 accessions), France (3 accessions), Belgium (2 accessions) and Portugal (2 accessions). The molecular results confirmed the morphological observation of a rather limited degree of variation between the accessions from Benelux/France. By combining all available sets of data (with an emphasis on RAPD-PCR results), eight major 'type classes' were constructed, two of them consisting of only one Portugese accession each(type classes VI/VII, see Table 4). These type classes contain subtypes with up to 15 diploid accessions exhibiting identical morphological, isozyme and RAPD patterns, with one RAPD pattern (A) being common to 49 accessions. They probably will permit a distinct reduction in accession numbers of the respective types, while additional research on not yet classifiable accessions might reduce the number of different types even further.

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Table 1. New *Brassica* accessions† at the IPK Genebank at Gatersleben since 1994 (continued from Gladis and Hammer 1995)

Year	Collecting mission to	No. accessions	*Brassica* species
1994		**12**	
	Canary Islands	5	*B. oleracea*
	Italy	5	*B. oleracea*
		1	*B. rapa*
		1	*B. tournefortii*
1995		**14**	
	Albania	1	*B.* sp.
	Spain	1	*B.* sp.
	Italy	2	*B. oleracea*
		1	*B. rapa*
	Uzbekistan	9	*B. rapa*
1996		**2**	
	Croatia	1	*B. sp.*
	Israel	1	*B. tournefortii*

† New accessions from institutions/persons: 1995 – 99 new accessions, 1996 – 41 new accessions.

Table 2. Number of *Brassica* accessions in German genebanks†

Brassica species	Total	BAZ/ Braunschweig	IPK/ Gatersleben	IPK/ Malchow
B. alboglabra Bailey	15	–	13	2
B. balearica Pers.	1	–	1	–
B. bourgeaui (Webb.) O. Kuntze	2	–	2	–
B. carinata A. Br.	155	115	37	3
B. cretica Lam.	3	–	3	–
B. elongata Ehrh.	8	–	7	1
B. fruticulosa Cyr.	10	–	9	1
B. gravinae Ten.	5	–	5	–
B. incana Ten.	3	–	3	–
B. juncea (L.) Czern.	287	93	131	63
B. macrocarpa Guss.	7	–	7	–
B. napus L.	1343	314	198	831
B. narinosa Bailey	5	–	1	4
B. nigra (L.) Koch	177	97	46	34
B. oleracea L.	1587	393	950	244
B. oxyrrhina (Coss.) Coss.	4	–	2	2
B. perviridis Bailey	2	–	2	–
B. rapa L. em. Metzg.	860	200	450	210
B. repanda DC.	2	–	2	–
B. rupestris Raf.	6	–	6	–
B. souliei (Batt.) Batt.	4	–	3	1
B. scopulorum (Pomel) Batt.	1	–	1	–
B. tournefortii Gouan	10	–	10	–
B. villosa Biv.	6	–	6	–
B. sp.	205	–	150	55
Total	**4708**	**1212**	**2045**	**1451**

† BAZ/Braunschweig: Federal Centre for Breeding Research on Cultivated Plants, 38116 Braunschweig, Germany (Data from L. Frese)

IPK/Gatersleben: Institute of Plant Genetics and Crop Plant Research (IPK) – Genebank, 06466 Gatersleben, Germany (Data from H. Knüpffer)
IPK/Malchow: IPK–Genebank, Malchow External Branch Station, 23999 Malchow/Pöl, Germany (Data from E. Willner).

Table 3. Number of accessions of *Brassica* species in German genebanks with respect to the region of origin

***Brassica* species**	**Species total**	**Genebank**	**Genebank total**	**Europe**	**Asia**	**Africa**	**North America**	**South/Central America**	**Australia/ N. Zealand**	**Unknown origin**
carinata	155	BAZ/Braunschweig	115	0	2	112	0	0	0	1
		IPK/Gatersleben	37	1	0	32	0	0	0	4
		IPK/Malchow	3	0	0	1	0	0	0	2
juncea	287	BAZ/Braunschweig	93	37	20	3	5	0	0	28
		IPK/Gatersleben	131	20	37	0	5	7	0	62
		IPK/Malchow	63	37	13	0	5	0	0	8
napus	1343	BAZ/Braunschweig	314	204	14	2	12	1	5	76
		IPK/Gatersleben	198	91	26	5	1	1	5	69
		IPK/Malchow	831	747	19	0	21	0	13	31
nigra	177	BAZ/Braunschweig	97	42	14	2	1	1	0	37
		IPK/Gatersleben	46	28	0	2	0	0	0	16
		IPK/Malchow	34	17	0	0	0	0	0	17
oleracea	1587	BAZ/Braunschweig	393	259	69	3	7	1	1	53
		IPK/Gatersleben	950	641	22	18	23	4	2	240
		IPK/Malchow	244	202	5	0	8	0	1	28
rapa	860	BAZ/Braunschweig	200	93	43	0	8	3	0	53
		IPK/Gatersleben	450	114	132	14	4	10	1	175
		IPK/Malchow	210	102	52	0	10	0	2	44
Brassica	352	BAZ/Braunschweig	0	0	0	0	0	0	0	0
spp.		IPK/Gatersleben	233	63	91	8	1	3	0	67
		IPK/Malchow	66	58	5	0	0	0	0	3
Total			**4708**	**2756**	**564**	**202**	**111**	**31**	**30**	**1014**

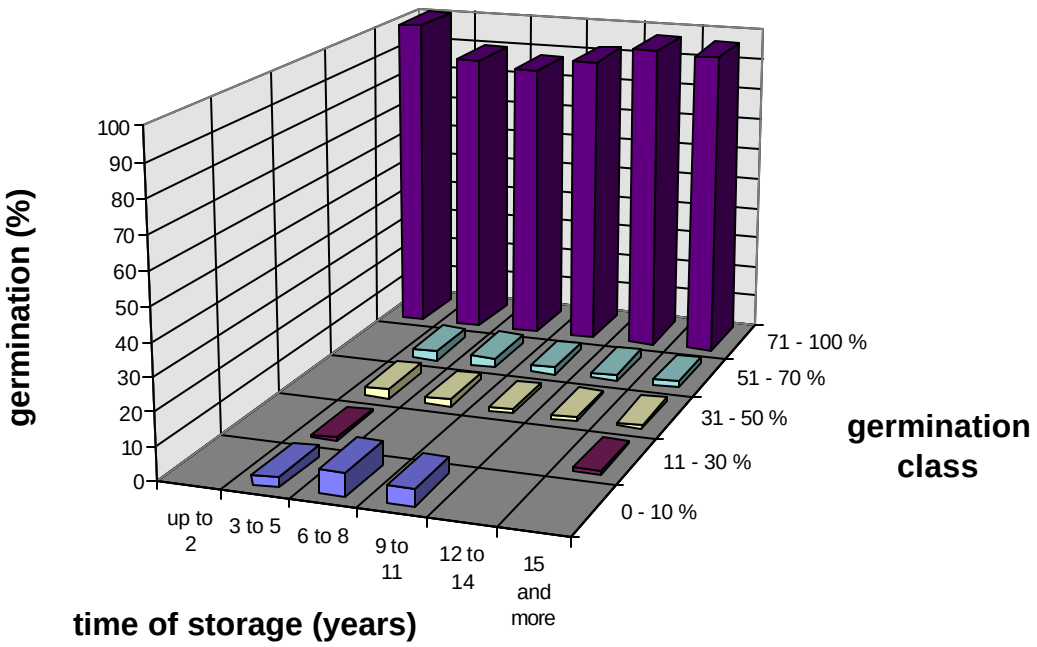

Fig. 1. Germination classes of *Brassica spp.* seeds after different years of storage, based on data from 1834 germination tests.

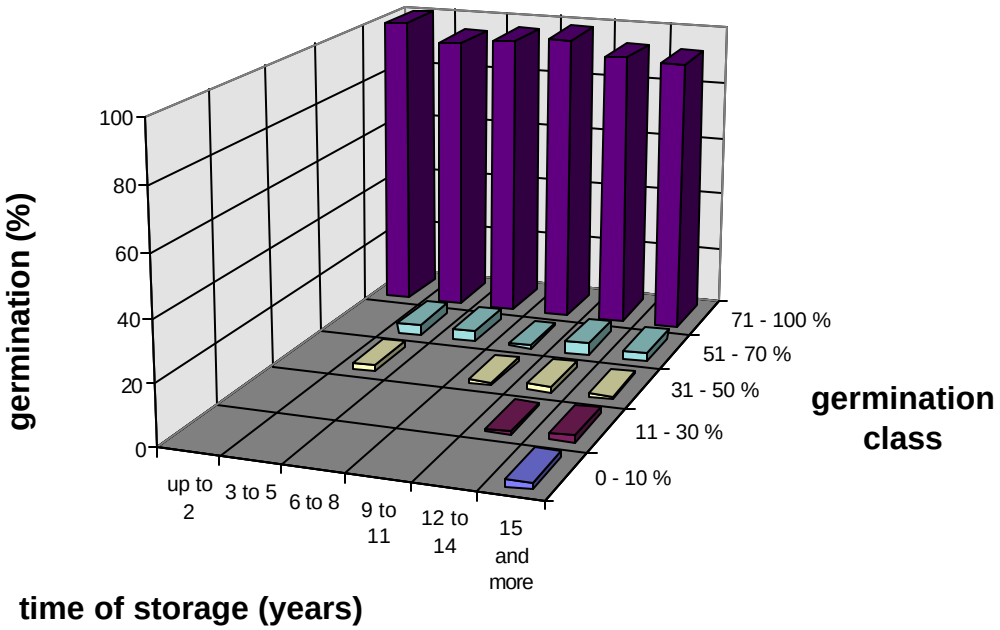

Fig. 2. Germination classes of seeds from cultivated *Brassica oleracea* accessions after different times of storage (928 seed samples tested).

Perspectives for activities regarding *Brassica* germplasm conservation and utilization in Greece

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Activities regarding complementary collections and evaluation were at standstill over the last 2 years. The main reason for this was the expectations for an EU-supported *Brassica* Genetic Resources Project where such activities would be coordinated at a European level. However germplasm collecting is not favoured in the EC 1467 programmes, so this activity could be realized only through specific national action or possibly through the ECP/GR project, as it was proposed in the *Brassica* Genetic Resources Workshop of 1994 in Lisbon. The EC project could assist in the regeneration and evaluation of the conserved germplasm.

It is rather certain that there will be no new call for proposals in 1997 due to cuts in the respective EU budget. The programme is hopefully expected to resume action in 1998. However, the possibility of success for a project is very slim since very few programmes are eventually funded. Having this in mind the working group should direct its efforts for collaboration to low cost activities supported by respective national funding.

So, in the absence of new collections, the Greek Gene Bank (GGB) still maintains in its facilities 169 accessions of *Brassica*, of which 43 belong to the wild relative species *Brassica cretica* and the remaining 126 to the cultivated species *Brassica oleracea.* Priority for new collections is given primarily to the Ionian islands and the coast of north-western mainland and this activity is envisaged for 1997.

As regards the status of conservation and diversity, limited scale exploratory regeneration in 1996 has shown that the germplasm has maintained high levels of viability on our medium-term storage (0-5ºC and 20-30% air humidity) or under the sealed packaging in glass tubes made by their collector Dr Gómez-Campo. The regenerated accessions expressed extreme variation in morphology, flowering initiation and other agronomic characteristics, giving an indication of the high hidden potential for further selection and breeding.

The Greek Gene Bank has acquired isolation cages for about 40 populations which can be used for regeneration of the *Brassica* germplasm, so a major constraint to their regeneration and evaluation has been removed.

Table 4. Characterization of *Brassica oleracea* L. var. *ramosa* DC. accessions[†]

Type class	RAPD type	Chromosome no.	Isozyme type	Morphotype	Country of origin	Accession no.[‡]
Ia	A	18	1	1	NL	03, 06, 12, 17, *23*, 27, 32, 34, 35, 36, 37, 39, 41, 45, 49
			1*	1	NL	19
			1	2	NL	13
			2	1	NL	05, 38, 44
			2?	1	*F*, NL	57, 58, *60, 61, 62,* 63
			2?	2	NL	52, 54, 56, 59
			3	1	NL	20, 22, 33
			3	2	NL	08
			3	3	NL	09
			7	1	NL	48
			8	1	NL	50
Ib	A	36	1*	1	*B*, NL	01, *07,* 18, 26, 28, 40, 43, 46
			1*?	2	NL	51
			4	1	NL	24
Ic	A	?	1?	–	NL	65
			2?	–	NL	64
II	B	18	5	1	NL	25
III	C	18	6	7	NL	29
IV	D	18	1	5	NL	15
	D	18	3	6	NL	16
V	E	?	2?	–	NL	66
VI	F	?	–	–	*P*	*67*
VII	G	?	–	–	*P*	*68*
VIII	–	18	–	1	NL	11, 21
	–	18	–	2	NL	14
	–	18	–	4	NL	10
	–	18	–	7	*B*	*30*
	–	18	–	8	NL	31
	–	18	2?	2	NL	53, 55

† Source: Zeven, A.C. *et al.* Are the duplicates of perennial kale (*Brassica oleracea* L. var. *ramosa* DC.) true duplicates as determined by RAPD analysis? (submitted for publication in Genetic Resources and Crop Evolution, July 1997). ‡ Source: A. Zeven, pers. comm.

Brassica germplasm in Italy

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Status of the collection at the Germplasm Institute

Cultivated species

Since 1971 the Germplasm Institute has collected 629 samples of *Brassica* from 16 countries (Table 1). In particular, 430 samples were collected in Italy, 109 come from Japan, 19 from China and a few samples from other countries. Collecting activity in countries others than Italy was carried out in collaboration with IBPGR and more recently with IPGRI. Among 16 species of *Brassica* those more represented are *B. oleracea* (308), *B. campestris* (93 + 17), *B. rapa* (82), and *B. napus* (18) (Table 2). The majority of the samples comes from Italy and Japan. In addition there are 72 samples not yet classified. The highest number of samples were collected from 1983 to 1986, within the framework of the EC Programme 0890 (contract CP15) and from 1992 to 1994, within the framework of an agreement with the Basilicata region (Table 3). Most of the samples collected since 1980 have been duplicated at IPK-Gatersleben, Germany and partly to other genebanks. Some specific types of *B. oleracea* have been distributed to seed companies.

In Bari the 629 seed samples of *Brassica*, dehydrated to 5-7% moisture content are stored under vacuum in special aluminium foil bags, at 0°C and 35-40% RH.

In 1982 and 1983, within the framework of an EC Research Programme 0890, in collaboration with NVRS/IVT (National Vegetable Research Station, Wellesbourne, UK/Instituut voor Tuinbouwplantenveredeling, Wageningen, The Netherlands) (contract: CP13), IDG participated in the collection of 362 varieties of *Brassica* from South Italy (Lazio, Marche, Campania, Sicily, Puglia and Marche). These samples are stored in Britain at Horticulture Research International (HRI, former NVRS) and have been recently duplicated at IDG in Bari.

Wild species

The IDG is also storing the duplicated collection of wild species of *Brassica* collected in 1984, 1985 and 1986 in Italy, Spain and France, within the framework of IBPGR (IPGRI), and in collaboration with other European countries (Snogerup *et al.* 1990). The original collection of 81 samples is stored in Madrid (Spain) at the Department of Biologia Vegetal, Universidad Politecnica. In particular, 43 samples were collected in south Italy in 1984, 25 samples in north Italy, south of France and Spain in 1985 and 13 samples in Sardinia and Corsica in 1986 (Table 4). As far as species are concerned, *B. montana* with 23 samples was the most representative and was collected in southern France, northern Italy (Tuscany and Liguria including small isles) and northeast Spain; *B. incana* with 22 samples was collected only in Italy and mainly in Campania, Sicily, Capri and Ischia and with only one sample in Tuscany; *B. insularis* with 13 samples was collected only in Sardinia and Corsica, *B. rupestris* with 10 samples was found only in Sicily, though some years later it was found and collected for the first time also in Calabria (Perrino *et al.* 1992); *B. villosa* also was found only in Sicily;

B. macropcarpa with 2 samples was found only in Favignana and Marettimo isles; *B. villosa* subsp. *drepanensis* and *B. tinei* with one sample each were found only in Sicily, while *B. pourretti* was found only in southern France.

Table 1. Samples of *Brassica* acquired by the C.N.R. - Germplasm Institute of Bari, Italy

Country of origin	1971-81	1982	1983	1984	1985	1986	1987	1988	1989	1990	1992	1993	1994	1995	1996	Total
Albania	–	–	–	–	–	3	–	–	–	–	–	5	7	2	–	17
Algeria	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
Bangladesh	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
Canada	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
China	–	5	7	–	–	6	–	–	–	–	–	–	1	–	–	19
South Korea	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
Egypt	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Ethiopia	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
France	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
Germany	–	–	–	–	–	9	–	–	–	–	–	–	1	–	–	10
Japan	–	–	–	–	–	109	–	–	–	–	–	–	–	–	–	109
Italy	101	1	144	21	17	7	2	19	7	6	11	78	7	3	6	430
Libya	–	2	7	–	–	–	–	–	–	–	–	–	–	–	–	9
Morocco	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
Tunisia	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Hungary	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
Total	**130**	**8**	**158**	**22**	**17**	**134**	**3**	**19**	**7**	**6**	**11**	**83**	**20**	**5**	**6**	**629**

Table 2. Accessions of *Brassica* stored at the CNR - Germplasm Institute of Bari, Italy

Origin[†]:	ALB	DZA	BGD	CAN	CHN	KOR	EGY	ETH	FRA	DEU	MAR	JPN	ITA	LBY	TUN	HUN	Total
Species																	
campestris	–	–	–	–	6	–	–	–	–	–	–	24	63	–	–	–	93
campestris+sp.	–	–	–	–	–	–	–	–	–	–	–	17	–	–	–	–	17
chinensis	–	–	–	–	1	–	–	–	–	–	–	2	–	–	–	–	3
fruticulosa	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
incana	–	–	–	–	–	–	–		–	–	–	–	1	–	–	–	1
japonica	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	4
juncea	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	6
macrocarpa	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	2
napus	–	–	1	–	1	1	–	–	1	2	–	2	2	8	–	–	18
nigra	1	–	–	–	–	–	–	1	–	–	–	–	1	–	–	–	3
oleracea	14	3	–	–	7	–	2	1	–	8	–	34	237	–	1	1	308
pekinensis	–	–	–	–	4	–	–	–	–	–	–	9	–	–	–	–	13
rapa	–	1	–	1	–	–	–	1	–	–	–	2	77	–	–	–	82
rupestris	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	3
villosa	1	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	3
Spp.	1	9	–	–	–	–	3	7	–	–	1	9	41	1	–	–	72
Total	**17**	**13**	**1**	**1**	**19**	**1**	**5**	**10**	**1**	**10**	**1**	**109**	**430**	**9**	**1**	**1**	**629**

[†] ALB = Albania; DZA = Algeria; BGD = Bangladesh; CAN = Canada; CHN = China; KOR = Republic of Korea; EGY = Egypt; ETH = Ethiopia; FRA = France; DEU = Germany; MAR = Morocco; JPN = Japan; ITA = Italy; LBY = Libya; TUN = Tunisia; HUN = Hungary. (ISO country codes).

Table 3. Accessions of *Brassica* acquired by the CNR - Germplasm Institute of Bari, Italy

Species	1971-81	1982	1983	1984	1985	1986	1987	1988	1989	1990	1992	1993	1994	1995	1996	Total
campestris	–	–	62	1	–	30	–	–	–	–	–	–	–	–	–	93
campestris+sp.	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1
campestris+sp.+*rapa*	–	–	–	–	–	16	–	–	–	–	–	–	–	–	–	16
chinensis	–	1	–	–	–	2	–	–	–	–	–	–	–	–	–	3
fruticulosa	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
incana	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
japonica	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	4
juncea	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	6
macrocarpa	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	2
napus	–	2	6	1	–	3	–	–	–	1	–	–	5	–	–	18
nigra	1	–	–	–	–	–	–	–	–	1	–	–	1	–	–	3
oleracea	75	3	74	12	9	48	3	10	4	2	4	50	10	3	1	308
pekinensis	–	2	2	–	–	9	–	–	–	–	–	–	–	–	–	13
rapa	2	–	13	–	4	3	–	9	3	2	6	32	2	1	5	82
rupestris	–	–	–	2	–	1	–	–	–	–	–	–	–	–	–	3
villosa	–	–	–	1	1	–	–	–	–	–	–	–	1	–	–	3
Spp.	52	–	1	3	3	9	–	–	–	–	1	1	1	1	–	72
Total	**130**	**8**	**158**	**22**	**17**	**134**	**3**	**19**	**7**	**6**	**11**	**83**	**20**	**5**	**6**	**629**

Table 4. Wild *Brassica* collected[†] in Italy, France and Spain, 1984-86 - CNR - Germplasm Institute, Bari, Italy

Country:	Italy								France		Spain	
Region:	Campania			Sicily		Sardinia	Tuscany	Liguria		Corsica		
Isle:		Capri	Ischia	Favignana	Marettimo							
Year:	1984	1984	1984	1984	1984	1984	1986	1985	1985	1986	1985	Total
Species												
B. montana	–	–	–	–	–	7	4	6	10	–	3	23
B. incana	9	4	2	6	–	7	1	–	–	–	–	22
B. insularis	–	–	–	–	–	7	–	–	–	6	–	13
B. rupestris	–	–	–	10	–	–	–	–	–	–	–	10
V. villosa	–	–	–	8	–	–	–	–	–	–	–	8
B. macrocarpa	–	–	–	–	1	–	–	–	–	–	–	2
B. villosa subsp. *drepanensis*	–	–	–	1	–	–	–	–	–	–	–	1
B. tinei	–	–	–	1	–	–	–	–	–	–	–	1
B. pourretti	–	–	–	–	–	–	–	–	1	–	–	–
Total	**9**	**4**	**2**	**26**	**1**	**7**	**5**	**6**	**11**	**6**	**3**	**81**

[†] Samples collected in collaboration with the Department of Crop Genetics and Breeding, Swedish University, Svalöv and the Departamento Biologia Vegetal Universidad Politecnica, Madrid, within the framework of IBPGR (IPGRI).

Details concerning the geographical distribution of the collected species, including description of the site of collection, latitude, longitude, altitude, size of the plant population, etc. can be found in the reports of the three collecting missions. The geographical distribution of the sites of collection is reported in Figures 1, 2, 3 and 4.

Recently the genebank in Bari has improved the conditions for regeneration activity, which are going to be fully used, thanks to funds coming from the Basilicata Region. Contacts just started with the Puglia Region may also provide new facilities for germplasm of vegetables, including *Brassica* ecotypes.

Status of the collections in other Italian centres

Only three other Italian centres confirmed that they maintain collections of *Brassica*. They are listed below with some information about their collections.

1. The Department of Botanical Science and Botanical Garden of the University of Palermo, holding more than 400 accessions of wild *Brassica* from different sites of Sicily and belonging to seven different species (*B. bivoniana* Mazzola et Raimondo, *B. villosa* subsp. *drepanensis* (Caruel) Damanti, *B. fruticulosa* Cyr., *B. macrocarpa* Guss., *B. nigra* (L.) Koch, *B. rupestris* Rafin., *B. souliei* (Batt.) Batt. An updated report with more details will be provided directly by experts from Palermo (Raimondo *et al.* 1991).
2. The Vegetable Crop and Floriculture Institute of the University of Catania, holding 192 accessions of *Brassica*. In particular the collection consists of the following taxa: *Brassica alba* (2), *B. campestris* (1), *B. fruticulosa* (5), *B. nigra* (4), *B. oleracea* var. *acephala* (9), var. *botrytis* (39), var. *gongylodes* (16), var. *italica* (82), *B. rapa* (20).
3. The Research Institute for Vegetable Crops of MiRAAF at Salerno, holding several lines and/or ecotypes of *B. oleracea*. In particular, the collection consists of 15 lines of Tardivo di Fano, one variety of Precoce di Jesi (including some breeding lines), one variety of Verde di Macerata (including some breeding lines), some lines of Cavolo Romanesco.

A more detailed report, regarding this point, may be provided later on when the investigation with other Italian centres, potential holders of vegetable crop collections, including botanic gardens, will be completed.

Research

Most of the research on *Brassica* in Bari deals with characterization of collected types. This activity is carried out in collaboration with IPK at Gatersleben and in the next future may include also the Vegetable Crop Institute of the University of Catania.

Recently, the IDG and the Botanical Garden of Palermo started a collaboration for investigating seed physiology during conservation in *Brassica villosa* subsp. *drepanensis* (Caruel) Damanti. In particular seeds from different natural habitats have been tested for their reaction to germinability under salt stress conditions. More information may be found in the report by experts from Palermo.

In collaboration with the Botanical Garden of the University of Palermo the IDG is going to start a project on *in situ* conservation of wild species of *Brassica*. Other experts from other countries may be involved in the project, though the main actors are expected to be from Palermo and Bari.

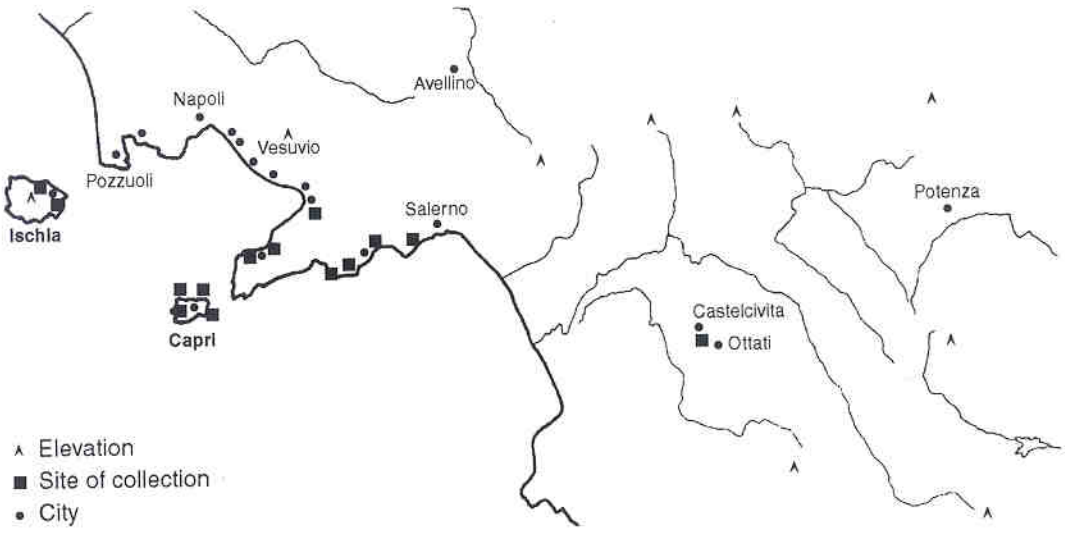

Fig. 1. Sites of collection of wild *Brassica* species in Campania, south Italy.

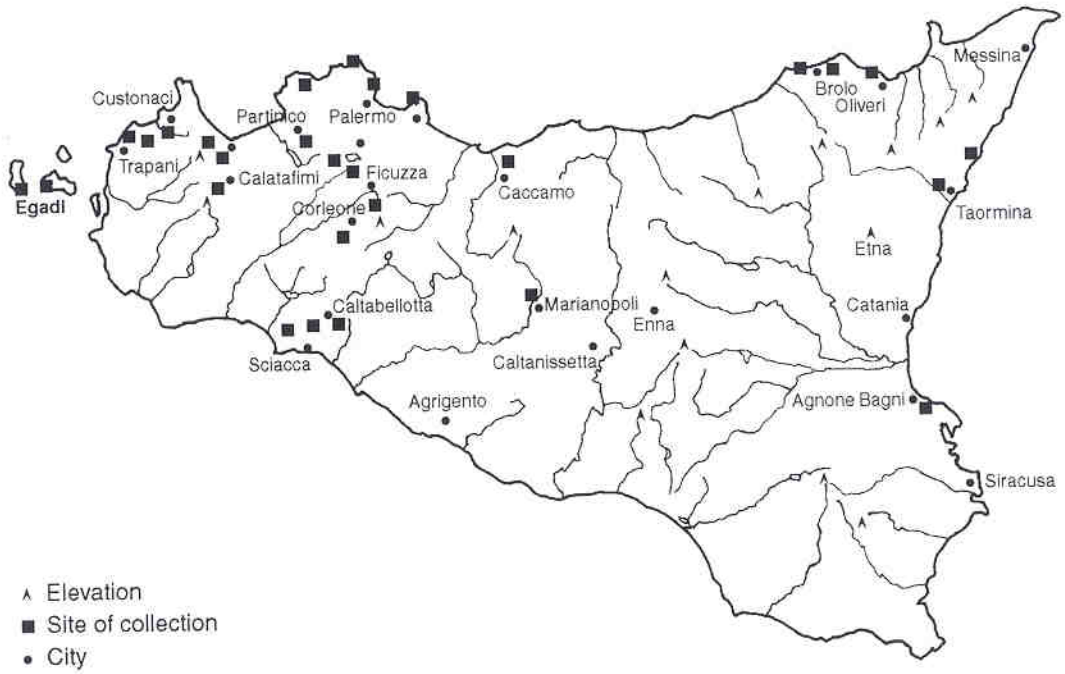

Fig. 2. Sites of collection of wild *Brassica* species in Sicily, south Italy.

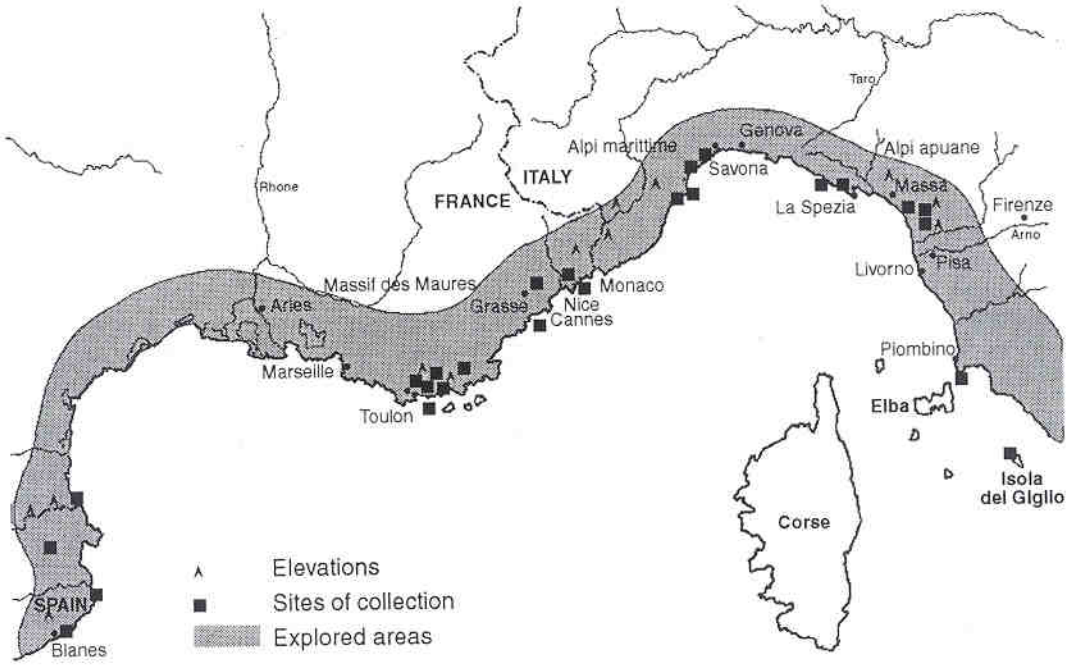

Fig. 3. Sites of collection of wild *Brassica* species in northern Italy.

Fig. 4. Sites of collection of wild *Brassica* species in Sardinia, Italy and Corsica, France.

In Italy research has been carried out at different levels. Information may be found concerning history, geographical distribution, economy and statistics (Bianco *et al.* 1988; Cafaro 1988; Miccolis 1990; Longarini 1992; Branca 1995; Branca and Iapichino 1997); morphology, biology, vernalization and other problems related to seeds (Magnifico *et al.* 1979; Noto *et al.* 1990; Perniola *et al. 199*2; Rivelli and Perniola 1992; Noto e Leonardi 1995); cultivation practice dealing with cultivar choice and competition (Dellacecca *et al.* 1988, 1990; Noto 1988; Paratore and Ruggeri 1988; Palumbo and D'Amore 1992; Ruggeri 1996); propagation and growth density (Damato and Bianco 1988, Damato *et al.*,1990); water irrigation (Tarantino and Rubino 1989; Rubino 1988); weed control (Montemurro and Sarli 1988); description of cultivation and methodology (Dellacecca *et al.* 1992; Giammetta 1988); diseases dealing with bacteria and fungi (Grasso 1988); animals (Di Vito 1988; Ragusa and Russo 1988), physiology (Magnifico *et al.* 1988); chemical composition (Magnifico and Elia 1988); post-harvest physiology and methods of conservation (Damato *et al.* 1990; Gorini 1988; Leoni *et al.*,1988); transformation and utilization as a forage (Celi *et al.* 1988) and flowering (Morone Fortunato *et al.* 1988).

It seems that important fields of research, like taxonomy, varietal description, plant breeding, soil fertilization, agricultural machinery, phytoregulators, pharmacology, biochemistry and therapeutic properties are not often considered by Italian scientists. Some of these fields have not received much attention. Flowering and ontogenesis receive greater study by scientists from other countries.

Although Italy is one of the most important geographical areas within the centre of origin of *B. oleracea*, which represents one of the most important economic vegetable crops, there are relatively few and no full-time *Brassica* specialists.

Future plans

As mentioned, the IDG future programme, with minor or slight changes, remains the one prepared and presented in 1987 as a 5-year project. It concerns exploration, collecting, multiplication and evaluation of cultivated species of the *B. oleracea* group.

The IDG, in collaboration with other national institutions, will be involved in establishing strategies for *in situ* conservation of wild species of *Brassica* and on-farm conservation of local types of cultivated *Brassica* species of the *oleracea* group.

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Current status of the CGN Cruciferae collection

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The collection

The CGN Cruciferae collection consists of 1562 accessions (November 1996). An overview of the collection is given in Table 1. The main changes compared to the situation as reported during the second working group meeting in November 1994 in Lisbon (Boukema 1995) is that 279 accessions (mainly *Brassica napus*, *B. oleracea* and *Raphanus sativus*) entered the collection after regeneration.

All information on the collection is available in any form, i.e. printed or in computer files. The passport data also can be found on the homepage of CGN on the Internet (http://www.bib.wau.nl/cgn/).

Regeneration

All material included in the CGN collection has been regenerated and fulfils our standards regarding quality and quantity (germination > 80%, > 4500 seeds). Some material no longer fulfilling these standards and some material not yet included in the collection needs to be regenerated.

Storage

The seeds are dried until a seed moisture content of about 5% is reached. The seed samples are packed in laminated aluminium foil bags and stored at –20°C for long-term storage. User samples are stored at 4°C.

Safety-duplication

About 85% of the collection is duplicated at the Genetic Resources Unit of HRI, Wellesbourne, United Kingdom. The remaining part, consisting of material regenerated during the last 2 years, will be sent to HRI before January 1997.

Characterization/evaluation

There are no major changes from the situation reported in November 1994 (Boukema 1995).

Utilization

In the last 2 years, 1734 seed samples of the collection were distributed for utilization to breeding companies and institutions.

Research

Some 86 accessions of *Brassica oleracea* belonging to the core collection developed for the EU project entitled "The location and exploitation of genes for pest and disease resistance in European genebank collections of horticultural brassicas" (Leckie *et al.* 1996) are screened by the project team on downy mildew (*Peronospora parasitica*), white blister (*Albugo candida*) and cabbage aphid (*Brevicoryne brassicae*).

This core collection has been used also by CGN to investigate if differences between cultivar groups can be revealed with the help of isozyme markers. So far

no clear results were obtained. Molecular research was started in *B. oleracea* to investigate the diversity in Dutch white cabbage.

Table 1. Number of accessions (CGNnrs) per cultivar group

Scnr	Cultivar group	CGNnrs
0702	*Brassica oleracea* group borecole	47
0704	*Brassica oleracea* group marrowstem kale	7
0707	*Brassica oleracea* other or unspecified kales	8
0708	*Brassica oleracea* group chinese kale	19
0711	*Brassica oleracea* group white cabbage	148
0712	*Brassica oleracea* group pointed headed cabbage	24
0713	*Brassica oleracea* group red cabbage	31
0714	*Brassica oleracea* group savoy cabbage	50
0717	*Brassica oleracea* group brussels sprouts	54
0718	*Brassica oleracea* group kohlrabi	19
0719	*Brassica oleracea* group cauliflower	184
0720	*Brassica oleracea* group broccoli	3
0723	*Brassica oleracea* other or unspecified	1
0724	*Brassica* wild species : 2*n*=18	5
0726	*Brassica napus* group fodder rape	32
0727	*Brassica napus* group swede	7
0728	*Brassica napus* group winter (oilseed) rape	123
0729	*Brassica napus* group spring (oilseed) rape	5
0730	*Brassica napus* other or unspecified	10
0734	*Brassica rapa* group fodder turnip	167
0735	*Brassica rapa* group vegetable turnip	45
0736	*Brassica rapa* group spring turnip (oilseed) rape	10
0737	*Brassica rapa* group winter turnip (oilseed) rape	13
0738	*Brassica rapa* group pe tsai (chinese cabbage)	45
0739	*Brassica rapa* group pak choi	16
0740	*Brassica rapa* group mizuna	3
0741	*Brassica rapa* group komatsuna	3
0742	*Brassica rapa* group yellow sarson	2
0743	*Brassica rapa* group broccoletto	21
0747	*Brassica rapa* group turnip greens	2
0748	*Brassica rapa* other or unspecified	13
0752	*Brassica juncea* group oilseed	18
0753	*Brassica juncea* group vegetable	4
0757	*Brassica carinata*	108
0761	*Brassica nigra* group black mustard	24
0764	*Brassica* unspecified	7
0766	*Brassica* other wild species	1
0770	*Sinapis alba* wild	7
0771	*Sinapis alba* group white mustard	44
0776	*Raphanus sativus* group radish	60
0777	*Raphanus sativus* group giant radish	70
0778	*Raphanus sativus* group fodder radish (oilseed)	43
0779	*Raphanus sativus* group mougri *(caudatus)*	2
0780	*Raphanus sativus* other or unspecified	28
0784	*Camelina sativa*	1
0786	*Eruca sativa*	7
0796	*Xbrassicoraphanus* group radicole	5
0797	*Xbrassicoraphanus* group raparadish	6
0798	Other cruciferae (excluding ornamentals)	3
0799	Cruciferae unspecified	3

The results of research on the verification of duplication using isozyme markers, as reported in the last meeting of November 1994, were published in Genetic Resources and Crop Evolution (Hintum *et al.* 1996).

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Status of the *Brassica* germplasm in the collection of vegetable crops in Poland

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The collection

The conservation work concerning vegetables crops from the genus *Brassica* is conducted in the Research Institute of Vegetable Crops in Skierniewice within the programme of the Polish Gene Bank. The collection of *Brassica* germplasm consists of 462 accessions of 16 species. A review of the current status of the *Brassica* collection is given in Table 1. This material includes advanced, old and obsolete cultivars and landraces, breeding materials mainly as sources of disease resistance (clubroot, downy mildew), sterility and self-incompatibility.

Collecting missions

In Poland it is still possible to find old cultivars and landraces of brassica vegetables (white and red cabbage, rutabaga, turnip, mustard). Sources of this germplasm are home gardens mainly in the north, northeastern, eastern, south and southeastern regions of Poland.

Since 1991, nine collecting missions have gone to these regions. Eight of these missions collected 22 *Brassica* accessions. In addition, two missions abroad brought back eight accessions. Collected materials are shown in Table 2.

Characterization and evaluation

All accessions of the mentioned collection are documented for passport data. Passport data are stored in dBase files in the Polish Genebank in Radzików and a copy is prepared for integration into the Bras-EDB.

About 50% of collected materials have been characterized and/or evaluated for different morphological, agronomic traits (Table 1). Evaluation data are stored in separate database files according to type of evaluation, year and experiment. The data include:

- 72 characters of white cabbage (RWPG descriptors) and 43 characters of white cabbage (UPOV and COBORU descriptors)
- 31 characters of Brussels sprouts, 29 of broccoli, 30 of cauliflower, 33 of kohlrabi according to UPOV descriptor lists.

Part of the evaluation data need to be computerized. The accessions of white and red cabbage have been characterized according to the RWPG (Council for Mutual Economic Aid) descriptor lists.

Other species of *Brassica oleracea* have been evaluated according to UPOV descriptor lists (and partially descriptors elaborated in POLSKV).

Part of the materials have been tested for resistance to downy mildew (110 accessions) and clubroot (80 accessions). The results of these tests are given among evaluation data.

Storage and safety-duplication

The collected materials are stored in the Central Gene Bank storage in Radzików in glass jars (twist-type). The seed samples are dried to a seed moisture content of about 5-7%. The seeds are stored at –18°C for long-term storage and at –4°C for short-term storage. Up to now *Brassica* accessions are not duplicated elsewhere.

Table 1. Status of *Brassica* germplasm in the Research Institute of Vegetable Crops, Skierniewice (POLSKV), November 1996

Polish name	Name	Total	Passport data	Evaluation / Characterization	Seed in long-term storage
Kapusta biala	white cabbage	76	76	70	76
Kapusta czerwona	red cabbage	7	7	3	7
Kapusta brukselska	Brussels sprouts	19	19	5	19
Kapusta wloska	Savoy	15	15	5	15
Kapusta pekinska	Chinese cabbage	55	55	25	55
Kalafior	cauliflower	160	160	100	160
Kalarepa	kohlrabi	8	8	8	8
Brokul	broccoli	14	14	14	14
Brukiew	rutabaga	6	6	–	6
Karpiel	rutabaga	3	3	–	3
Jarmuz	kale	3	3	–	3
Rzepa	turnip	26	26	–	26
Gorczyca	mustard	7	7	–	7
Rzodkiewka	small radish	39	39	–	39
Rzodkiew	radish	18	18	–	18
Rzezucha	watercress	1	1	–	1
Inne kapustne	other brassicas	5	5	–	5
Total		**462**	**462**	**230**	**462**

Table 2. *Brassica* accessions collected during missions organized 1991-95 , Skierniewice 1996

Year	Organization	Area	Collected germplasm	No. of access.
1991: Sept - Oct	MAFF-Japan POLSKV-Poland	around S/E/N parts of Poland	mustard white cabbage turnip rutabaga	3 2 1 1
1993: August	POLSKV	Province Przemysl (Jaroslaw, Walawa, Bolestraszyce) Province Krosno (Nehrybka, Huwinki, Arlamowo, Rybotycze, Dobryn, Folusz) Province Nowy Sacz (Debno, Podrzecze, Dominikowice)	turnip	1
1993: November	POLSKV	Province Kielce (Bieliny, Slupia, Lagów, Stryczowice) Province Tarnobrzeg (Opatów, Czerwona Góra) Province Przemysl (Niziny, Walawa)	turnip	2
1994: October	POLSKV	Province Suwalki (Punsk, Szypliszki, Poszeszupie, Rutka Tartak, Szlinokiene, Baranowo Pobadzie	white cabbage mustard curly kale rutabaga	5 1 1 2
1994: Sept	IPGRI, IPK, POLIHAR	Albania	white cabbage	4
1995: August	POLIHAR	Tatry - Slovakia	white cabbage rutabaga	2 2

1995: October	POLSKV	Province Biala Podlaska	white cabbage	2
			watercress	1

Table 3. Number of *Brassica* accessions distributed since 1991-96 (Skierniewice 1996)

		Number of accessions distributed	
Year	**Species**	**Breeding company, Institute, University**	**Abroad**
1991	cabbage	15	–
	Brassica campestris	2	–
	Chinese cabbage	6	–
	rutabaga	1	–
	turnip	15	–
	Total	**33**	–
1992	white cabbage	12	–
	Total	**12**	–
1993	red cabbage	–	2
	Brussels sprout	–	1
	Savoy cabbage	–	1
	kohlrabi	–	1
	cauliflower	–	1
	Chinese cabbage	8	–
	Total	**8**	**6**
1994	white cabbage	31	–
	turnip	3	–
	Chinese cabbage	4	–
	kohlrabi	1	–
	mustard	1	–
	Total	**40**	–
1995	kapusta (cabbage)	87	5
	brokul (broccoli)	7	3
	kalafior (cauliflower)	4	–
	kapusta pekinska (Chinese cabbage)	2	–
	kalarepa (kohlrabi)	2	–
	gorczyca (mustard)	5	–
	rzepak (rapeseed)	5	–
	rzepik (turnip rape)	2	–
	Total	**114**	**8**
1996	white cabbage	6	–
	Chinese cabbage	34	–
	turnip	2	–
	kapustne	3	–
	karpiel	3	–
	Total	**48**	–

Research

The germplasm is used in research projects which are conducted in the Research Institute of Vegetable Crops and in breeding companies to:

- obtain homogozygous dihaploid lines (using anther haploidization and now beginning to use microspore culture) of head cabbage and Brussels sprouts for the breeding of hybrids;
- breed cauliflower for resistance to downy mildew. The resistance was introduced from resistant cabbage and broccoli into susceptible cauliflower. Selection for resistance to downy mildew in this breeding material is continuing;

- breed cabbage for resistance to downy mildew;
- screen *Brassica* germplasm for resistance to clubroot.

Utilization

From 1991 until now, 269 seed samples of the collection have been distributed for utilization; 255 of these samples were used by Polish institutions (breeding company, institutes, universities) and 14 samples were sent abroad (Table 3).

Status of *Brassica* collections in Portugal

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National collections

The Portuguese *Brassica* germplasm is kept at three institutions (Table 1):

- Banco Português de Germoplasma Vegetal (BPGV) at Braga;
- Instituto Superior de Agronomia (ISA) at Lisbon;
- Horticulture Research International (HRI) at Wellesbourne.

Table 1. The Portuguese *Brassica* collections (1996)

	ISA	BPGV
B. oleracea (*tronchuda, acephala, capitata*)	496	613
B. rapa	100	250[†]
B. napus	42	
Raphanus sativus	3	
Total	**641**	**863**

[†] Some of these accessions are *B. napus.*

At ISA, germplasm collecting was carried out mainly in 1987 and 1988 (393 accessions). From 1988 to 1996 some material was collected every year (a total of 641 accessions in 1996). A joint mission with HRI in 1994 was realized in inland North Portugal (Trás-os-Montes). The ISA collection includes *Brassica oleracea* (496 accessions), *B. rapa* (100 accessions), *B. napus* (42 accessions) and *Raphanus sativus* (3 accessions).

The 393 accessions collected during 1987 and 1988 were packed in aluminium bags under vacuum in 1988. The rest of the accessions were kept in paper bags. Recently after germination tests, some of these last accessions were packed in aluminium bags under vacuum. The extra paper bags were all put together in large aluminium bags last year.

Only a few duplicates are kept at BPGV and at HRI. The quantity of seeds per each accession varies between 1 and 380 g and the germination rate of some accessions is not known.

Almost no regeneration has been done at ISA since the material was collected. In 1991 eight accessions were regenerated and in 1995, 40 accessions. The germination rate of some accessions is low and some do not germinate. In a recent test on 45 *Brassica oleracea*, 45 *B. rapa* and 45 *B. napus*, 1 *B. oleracea*, 9 *B. rapa* and 5 *B. napus* did not germinate and the germination was very deficient in 4 *B. oleracea* and 11 *B. rapa*.

The ISA collection is basically considered a working collection.

At BPGV collecting was carried out from 1989 until 1996 (Table 2). Most of the collecting missions were not specific for brassicas, and were aimed at collecting maize and legumes. However a total of 863 accessions of brassicas has been collected so far, including *Brassica rapa* are *B. napus*. Almost one-half of the collection at BPGV has a duplicate at HRI. No regeneration has been carried out since the material was collected.

Table 2. Collecting missions carried out by BPGV

Year	*B. oleracea*	*B. rapa*[†]
1989	1	–
1990	124	27
1991/0	8	–
1991/A	2	1
1991/B	7	2
1991/C	74	7
1992/A	2	2
1992/B	5	2
1992/C FAV	14	15
1992/D RASD	71	50
1993/A/1	3	2
1993/A/2	–	1
1993/B	31	20
1993/C	1	1
1993/D	34	20
1993/E	9	–
1993/F	32	26
1994/A	15	6
1994/B	11	5
1994/C	36	10
1994/D	5	1
1994/E	2	3
1994/A ESAS	12	6
1994/B ESAS	17	10
1994/C ESAS	2	–
1994/D ESAS	4	6
1995/A	62	8
1995/B	3	4
1996	26	15
Total	**613**	**250**

[†] Some of these accessions are *B. napus.*

Collecting missions from 1994 to 1996

ISA did not carry out any special collecting mission during this period. Only nine accessions of *B. rapa*, 3 of B. *oleracea* and 1 of *R. sativus* were collected in 1996.

BPG carried out collecting missions in 1995 and 1996. In 1995 two collecting missions were made at Terras de Bouro (northwest Portugal) where 65 accessions of *B. oleracea* and 12 B. *rapa* were collected. In 1996 two collecting missions were made, one to Terras de Bouro and the other to Meda-Vila Nova de Foz Côa (inland center / north Portugal).

The Russian *Brassica* collection

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The *Brassica* collection of the N.I. Vavilov All-Russian Research Institute for Plant Industry contains 6879 accessions as shown in Table 1. The presence of this wide botanical diversity provides many possibilities for studying the biological composition of *Brassica* species and varieties. Our collection research pursues the following objectives.

- **To collect the seeds**
  The Genebank contains genetic resources from all five continents, but most of the accessions were collected in the territory of the former USSR and Europe. Unfortunately, the number of seed collecting expeditions at the present time has been reduced because of the economic situation.

- **To maintain the collection accessions in viable conditions and to provide long-term storage for them**
  This is one of the most important tasks at the moment; accessions are sown for regeneration every first or fourth year, depending on the crop class. For example, *Brassica rapa* is sown each year and *B. oleracea* every fourth year.

  The basic working collections of *Brassica* crops are stored in St Petersburg in laminated aluminium bags at room temperature. In the National Seed Storage Facility located on the Kuban Experiment Station, the seeds are preserved in medium-term storage at a temperature of 4°C.

  In 1995 IPGRI provided assistance to VIR's Genebank to acquire upgraded refrigeration equipment for long-term storage where the seeds are preserved at a temperature from -15 to -20°C.

Table 1. Collections of *Brassica* spp. in VIR and their documentation

	Number of accessions	
Species	**in collection**	**passport data**
B. napus L. subsp. *oleifera*	770	770
var. *napobrassica* (L.) Rehb.	265	265
B. campestris L.	310	310
B. juncea L.	1139	1114
B. carinata A. Braun	41	41
B. nigra (L.) Koch	35	35
B. oleracea L.		
var. *capitata* L.	1760	927
var. *botrytis* L.	947	500
var. *sabauda* L.	188	110
var. *gemmifera* DC.	235	26
subsp. *oleracea*	206	117
var. *gongylodes*	183	110
B. chinensis Jusl.	84	34
B. rapa L.	386	386
B. rapa var. *pekinensis* (Lour) Rupr.	330	136
Total	**6879**	**4881**

- **To study the *Brassica* collection species in order to identify the promising materials for breeding and pre-breeding of the most important commercial traits in different ecological regions of Russia.**
  The collections are studied and regenerated in experimental stations which are situated within the country's different ecogeographical zones. There are eight experimental stations where studies and regeneration of *Brassica* crops are carried out using the same standardized research methods.
  We have very good collaboration activities with the Japanese 'Takii' and with the Dutch 'Royal Sluis' seed companies which provide us with new regenerated materials.

- **To organize the computerized database in order to make the collection data immediately available for breeding centres and to facilitate data exchange with the other genebanks.**
  The activities planned for 1997-1998 include the updating of the passport data and evaluation data and the storage of seeds into refrigerators for long-term conservation in St Petersburg.

List of curators and crops:

Prof. V.I. Burenin	*Brassica napobrassica, B. rapa*
Dr A. Artemjeva	cabbage collection
Dr O. Dzjuba	*B. juncea, B. carinata, B. nigra, B. campestris*
Dr A. Dubovskaya	*B. napus* subsp. *oleifera, B. napus*

Status of the *Brassica* collections in Slovakia

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The Research and Breeding Institute for Vegetable and Special Plants has a long history. The first research works were registered in 1926 with the acclimatization of exotic crops. After many changes the principal activities were directed to vegetable crops.

At present the Institute is the only station in Slovakia for research, breeding and genetic resources for vegetables and medicinal plants. Research work dealing with cultivation technologies, breeding, multiplication of biological material, collecting, study and conservation of vegetable and medicinal plants is carried out by the Institute. Additional functions are special consultancies, preparation of projects, analysis of trends in vegetable production, and commercial activities with seeds and planting material.

Vegetable crops in Slovakia represent the largest group of cultivated crops. The Slovak list of registered cultivars comprises 48 species of vegetables with 747 cultivars. The genetic variability of the material in collection can be used by breeders to meet the distinct demands of cultivation conditions and methods. In the framework of the Ministry of Agriculture the genetic resources of vegetable and medicinal plants were entrusted to the Research and Breeding Institute for Vegetable and Special Plants in Nové Zámky.

Collecting, study and conservation of vegetable and medicinal plants genepool are permanent activities of the Institute. In 1995-96, 31 vegetable species and 14 medicinal plant species represented by 482 samples were evaluated there. The number of stored samples is 510.

The *Brassica* collection in Slovakia is not large. After the privatization of the breeding station at Kvetoslavov more than 452 accessions were lost (Table 1). In 1995 the Institute in Nové Zámky started with the study, gathering and preservation of *Brassica* accessions with 40 samples (Table 2) and in 1996 there were 70 samples. The total number of accessions for the genus *Brassica* in Slovakia was 175 (Table 3). The new genebank in the Research Institute of Plant Production in Piestany is now open. This will provide long-term storage structures and increase the numbers of collections in the country.

Table 1. *Brassica* genetic resources collections in Slovakia - 1990

Genus	Species	Variety	Number	Holding institute
Brassica	*oleracea*	*gongylodes*	210	Breeding station, Kvetoslavov
		botrytis	195	
		capitata	47	
Total			**452**	

Table 2. *Brassica* genetic resources collections in Slovakia - 1995

Genus	Species	Variety	Number	Holding institute
Raphanus	*sativus*	*radicula*	15	Breeding station, Král'ová pri Senci
Brassica	*oleracea*	*gongylodes*	12	Breeding station, Kvetoslavov
Brassica	*oleracea*	*gemmifera*	7	Research and Breeding Institute for Vegetable and Special Plants, Nové Zámky
Brassica	*oleracea*	*gongylodes*	10	
Brassica	*oleracea*	*italica*	5	
Brassica	*oleracea*	*sabauda*	8	
Brassica	*rapa*	*pekinensis*	10	
Total			**67**	

Table 3. *Brassica* genetic resources collections in Slovakia -1996

Genus	Species	Subsp.	Number	Holding institute
Raphanus	*sativus*	*radicula*	15	Breeding station, Král'ová pri Senci
Brassica	*napus*	*napus* (spring)	26	Research Institute of Plant Production
Brassica	*napus*	*napus* (autumn)	56	
Crambe			8	
Brassica	*oleracea*	*capitata*	9	Research and Breeding Institute for Vegetable and Special Plants, Nové Zámky
Brassica	*oleracea*	*botrytis*	1	
Brassica	*oleracea*	*gongylodes*	19	
Brassica	*oleracea*	*italica*	8	
Brassica	*oleracea*	*sabauda*	20	
Raphanus	*sativus*	*radicula*	9	
Raphanus	*sativus*	*niger*	4	
Total			**175**	

Plant genetic resources activities in Turkey - brassicas

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Introduction

Plant genetic resources activities in Turkey started in 1964, and because of the importance given to plant genetic resources these studies were implemented within the framework of the National Plant Genetic Resources Research Project (NPGRRP) in 1976. The Aegean Agricultural Research Institute (AARI) has taken over all responsibility at national level in its capacity of 'project centre'. Cooperation with various institutions is arranged according to the principles of the National Code of Conduct on Collection, Conservation and Utilization (1992). All joint programmes are conducted on a project basis within agreements (Firat and Tan 1995).

Turkey is also a member of several international programmes working on plant genetic resources, such as the Global Plant Genetic Resources Commission of the Food and Agriculture Organization of the United Nations (FAO). Turkey has adhered to the Undertaking of Plant Genetic Resources; the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR); the West Asia-North Africa Plant Genetic Resources Network (WANANET) of the International Plant Genetic Resources Institute (IPGRI); the World Wheat Genetic Resources Networks and the *Beta* Genetic Resources Network.

The objectives of the NPGRRP are survey, collecting, conservation (both *ex situ* and *in situ*), documentation and evaluation of existing plant genetic resources and plant diversity in Turkey. Survey/collecting, multiplication/regeneration and utilization activities are organized by specialized groups for Cereals, Food Legumes, Forages, Industrial Crops, Vegetables, Fruit Trees, Ornamental Plants, Medicinal and Aromatic Plants, and Endemic Plants.

Turkey, one of the world's important centres for plant genetic resources, has a very rich flora with a remarkable diversity. This richness is due to Turkey being:

- part of the overlapping Vavilovian centres of plant diversity: Near Eastern and Mediterranean;
- a meeting place of three phytogeographical regions, namely the Euro-Siberian, Mediterranean, and Irano-Turanian regions;
- a bridge between Southern Europe and South-West Asia, which apparently served as a migration route;
- a centre of diversity for many genera and sections;
- a centre of origin for many cultivated plants and weeds in Europe;
- a country with a high level of endemism (Tan 1992).

Brassica genetic resources in Turkey

Ex situ conservation

Collections of seed material are preserved in cold stores of the genebank at AARI where the needs of long- and medium-term storage for base and active collections as well as short-term storage for working samples have been met thoroughly.

Additionally, in Ankara, there are storage facilities of base collections at the Field Crop Improvement Center for safety-duplicates.

Table 1. Conservation facilities in cold storage of the genebank at AARI

	Short term	Medium term	Long term
Temperature (°C)	+4	0	–20
Moisture content (%)	6 - 8	6 - 8	6
Storage volume (m^3)	80	240	80
Space availability	Yes	Yes	Yes
Container type	LAP	SCN	SCN
Viability monitoring	–	5-year intervals	10-year intervals

Distribution of brassicas in Turkey

Brassicas are widespread as wild, weedy and cultivated forms throughout Turkey (Davis 1965). The distribution of some *Brassica* and *Sinapis* species is shown in Table 2 and Figures 1, 2 and 3.

Table 2. *Brassica* and *Sinapis* in Turkey

Species	Distribution area	Form	Habitat
Brassica cretica	West and South Anatolia	Wild	Rocks, usually near sea level
Brassica elongata	Inner Anatolia	Wild, weedy	Dry rocky slopes, steppe and cultivated fields, 450-1700 m
Brassica deflexa	Southeast Anatolia	Wild, weedy	Dry rocky slopes and cultivated areas
Brassica nigra (=Sinapis nigra)	South, West and North Anatolia	Wild, weedy, cultivated	Waste places and fields in lowlands
Brassica tournefortii	West, South and Southeast Anatolia	Wild	Waste places, sea level, 1000 m
Sinapis alba	Scattered throughout Anatolia	Wild, weedy	Roadside and waste places, sea level, 1400 m
Sinapis arvensis	Widespread	Wild, weedy	Roadside and waste places, sea level, 1800 m

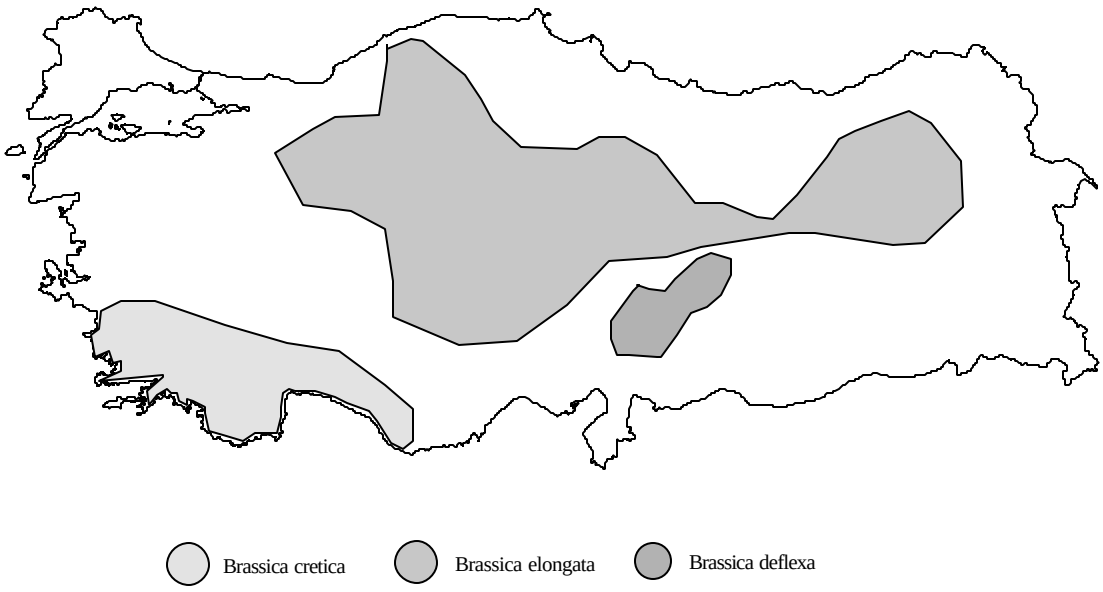

Fig. 1. Distribution areas of *Brassica cretica, B. elongata* and *B. deflexa* in Turkey.

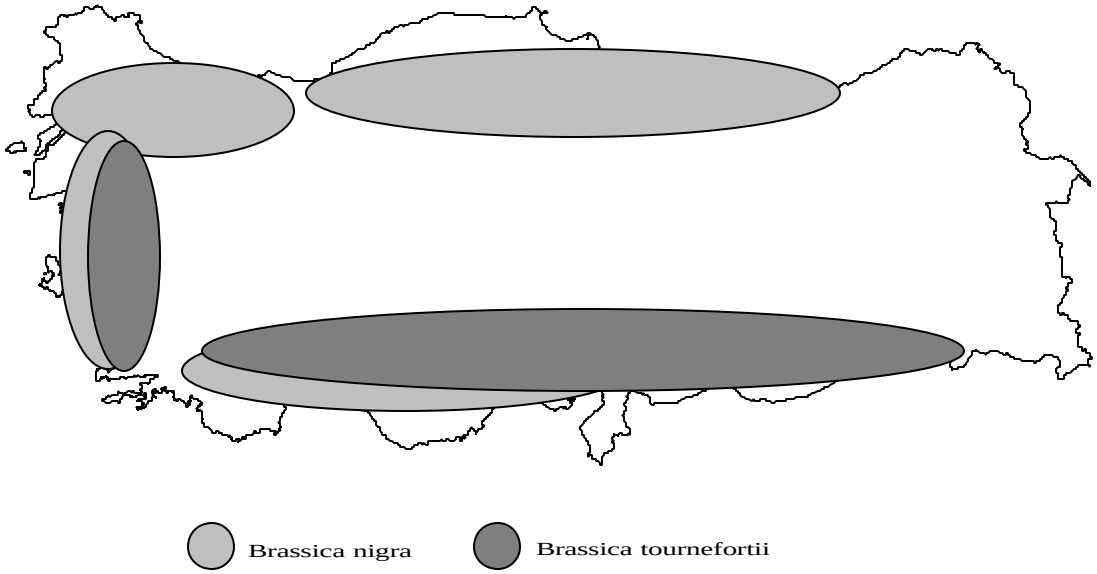

Fig. 2. Distribution areas of *Brassica nigra* and *B. tournefortii* in Turkey.

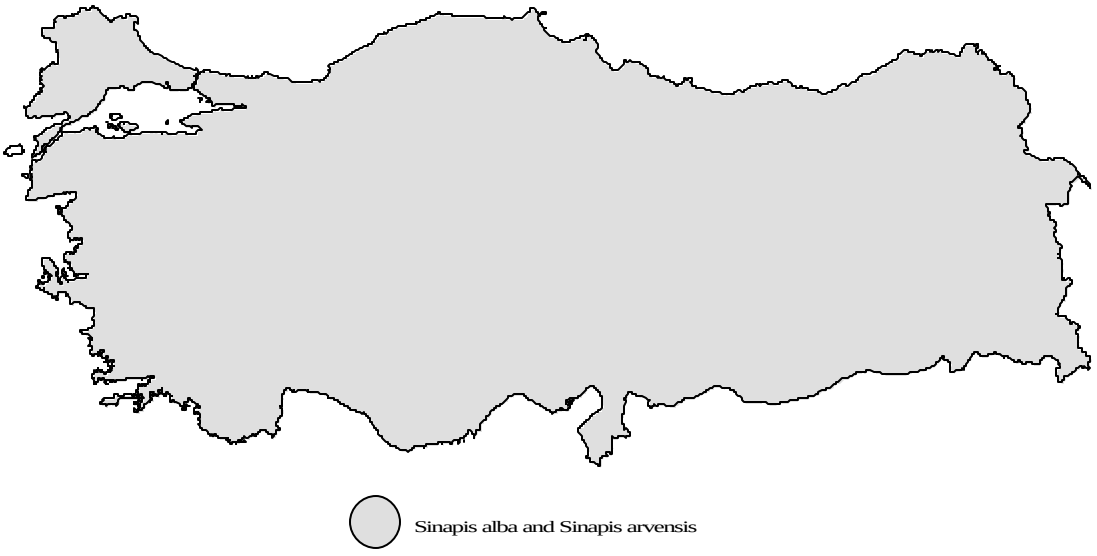

Fig. 3. Distribution areas of *Sinapis alba* and *S. arvensis* in Turkey.

Collections of brassicas and *Sinapis* in Turkey

Brassicas including *Sinapis* spp. collected since 1964 have exceeded 300. Systematic collecting and surveys are conducted by taking various priorities into account such as existing erosion factors, construction of dams and irrigation canals, grazing, land opening to industry, etc.

Wild, weedy and cultivated forms of brassicas, such as *B. oleracea, B. rapa, B. napus,* collected in the country, are shown in Table 3.

Collecting is still a priority in order to fill gaps of landraces found in Turkey, especially in the regions of Turkey which have never been visited for collecting.

Table 3. AARI *ex situ* collections of brassicas (including *Sinapis* spp.)

Species	Collected sites (provinces)[†]	No. of accessions
Brassica spp.	Diyarbakir, Kocaeli	4
Brassica cretica	Antalya, Aydin, Mugla,	6
Brassica nigra	Amasya, Giresun, Sinop, Kastamonu, Rize, Trabzon, Ordu, Samsun	65
Brassica oleracea	Istanbul, Sakarya, Bursa, Balikesir, Agri, Kars, Gümüshane, Sivas, Amasya, Erzurum, Giresun, Trabzon, Kars, Artvin, Gaziantep, Kastamonu, Mardin, Kayseri, Çorum, Sinop, Hatay, Urfa, Tokat, Ordu, Izmir, Rize, Aydin, Diyarbakir, Bitlis, Mus, Malatya, Çanakkale, Tekirdag, Kirklareli	198
Brassica rapa	Gaziantep, Mardin, Diyarbakir, Siirt, Bitlis, Elazig, Malatya, Kars, Giresun, Erzincan, Izmir, Kastamonu, Erzurum	24
Brassica napus	Erzurum, Tekirdag, Istanbul, Edirne, Kirklareli	15
Brassica campestris	Kars	1
Sinapis spp.	Kastamonu, Diyarbakir, Osmaneli, Hatay, Iskenderun, Agri, Bilecik	4
Sinapis nigra	Diyarbakir	1

[†] See Figures 4, 5 and 6.

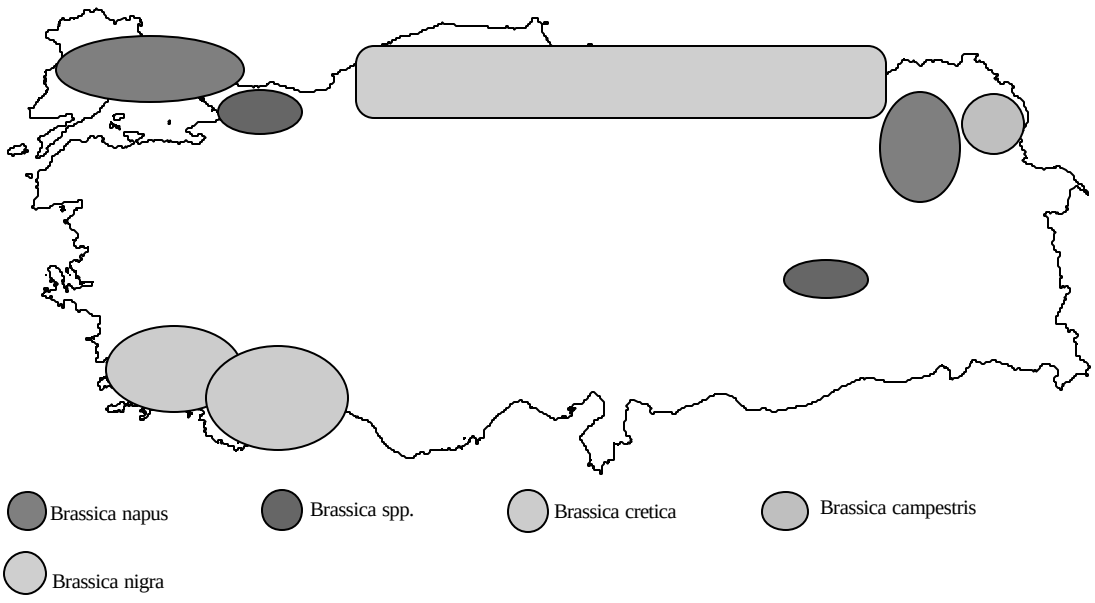

Fig. 4. AARI *ex situ* collections of *Brassica* spp., *B. napus, B. campestris, B. nigra* and *B. cretica.*

Multiplication/regeneration

Stored accessions with low germination rate, or decreased amount of active collections and insufficient amount of collection material are subject to the multiplication and regeneration programmes.

Evaluation and characterization

Evaluation and characterization studies will be conducted in the future.

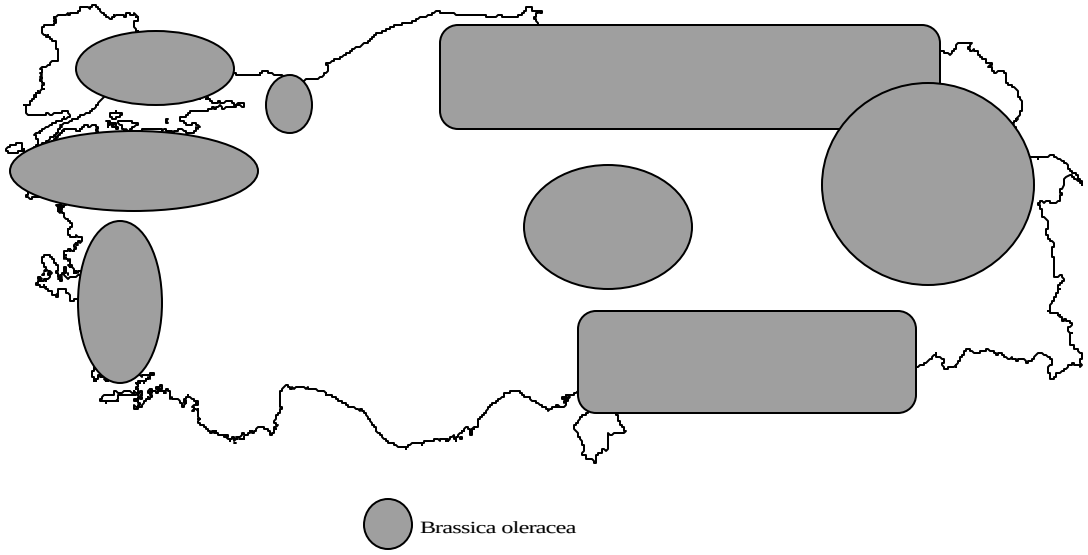

Fig. 5. AARI *ex situ* collections of *Brassica oleracea.*

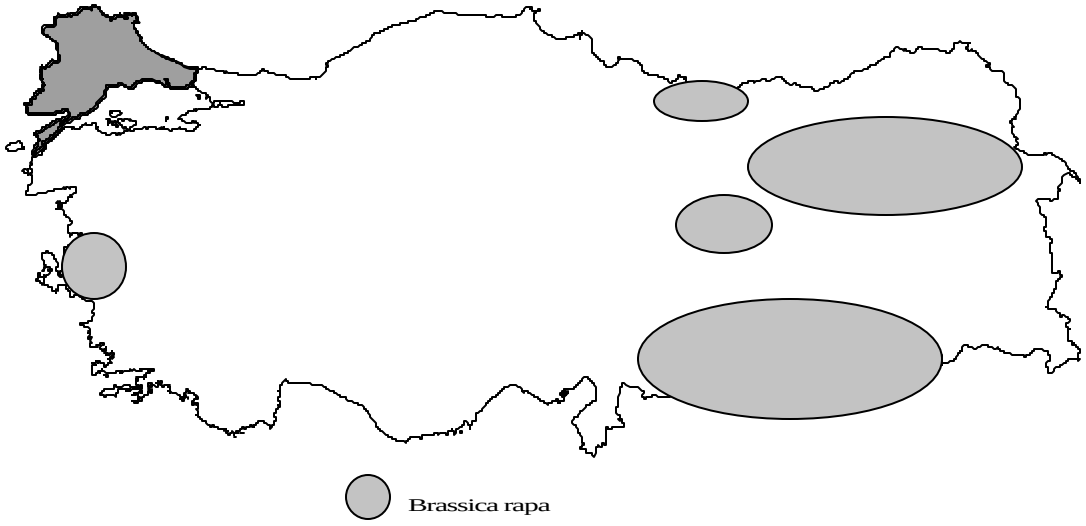

Fig. 6. AARI *ex situ* collections of *Brassica rapa.*

***In situ* conservation**

The *In situ* Conservation of Plant Genetic Diversity Project is an important part of the National Plant Genetic Resources Research Project, and has started in 1993 with the collaborative work between the Ministry of Agriculture and Rural Affairs (MARA), the Ministry of Forestry (MOF), and the Ministry of Environment (MOE). MARA and MOF are the implementing Ministries for the project.

The objectives of the project are as follows:

- to identify and establish the *in situ* conservation areas in Turkey for the protection of wild genetic resources originated from Turkey;
- to test and develop a new approach for conservation of genetic diversity;

- to provide sustainable *in situ* conservation of wild genetic resources of field and horticultural plants and forest trees.

Brassicas are given the secondary priority in the National Plan for *in situ* Conservation of genetic diversity

Status of central databases

The NPGRRP activity data are maintained in the databases created by dBase3+ and dBase4. Passport, collecting and storage information have already been computerized. The evaluation information is analyzed by using the multivariate analysis and statistical package programmes. The mapmaker package is also used for map production if the locations of collecting sites are recorded with the Global Positioning System (GPS). The Documentation Unit is responsible for the centralized Database of NPGRRP.

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Tan, A. 1992. Plant diversity and plant genetic resources in Turkey. Anadolu J. of AARI. 2 (1992):1-13 MARA.

UK collections of cruciferous crops

Dave Astley
HRI, Wellesbourne, UK

A number of cruciferous crop collections in the United Kingdom are listed in the Ministry of Agriculture, Fisheries and Food UK Review of *Ex situ* Plant Genetic Resources. Each collection has a different mandate with varying responsibilities as base and/or working collections.

The Genetic Resources Unit, Horticulture Research International Wellesbourne

The role of the HRIGRU has not changed markedly since the last ECP/GR *Brassica* report. The Unit is active in the conservation of a range of cruciferous crops in collaboration with the Centre for Genetic Resources the Netherlands (CGN). The current total of accessions for various taxa, an estimate of their availability and the extent of safety-duplication are given in Table 1. Availability of accessions is determined by seed numbers and/or percentage germination. Two strategic test collections, S-allele lines and European Clubroot Differentials, are maintained, documented and distributed to research workers. We have an agreement with CGN to store mutual safety-duplicates. A duplicate seed sample of new accessions is despatched as soon as possible after receipt, but accessions in store are being despatched routinely as and when the time permits. The percentage of the HRIGRU collection in safety-duplication with CGN has risen from 30% in 1994 to 49% currently.

Information relating to the collections is mainly passport stored using agreed ECP/IBPGR descriptors in dBase. Where characterization data exist the descriptors are a combination of IBPGR and UPOV. The HRIGRU and CGN have used the BrasEDB to develop a preliminary core collection in support of an EU research project.
Contact: Dave Astley.

The Scottish Agricultural Science Agency, East Craigs

SASA is responsible for testing UK applications for Plant Breeder's Rights and addition to the National List of candidate cultivars. The protocols used for testing crops are agreed by the Union for the Protection of Plant Variety Rights (UPOV). The Vegetable Crops Section maintains >1700 accessions including well-characterized reference sets of obsolete and current cultivars totalling 680 accessions of broccoli, Brussels sprout, cabbage, kale, radish, swede and turnip (Table 2). Seeds are stored in plastic bottles at -20^{0}C.
Contact: Niall Green.

The National Institute for Agricultural Botany, Cambridge

NIAB maintains a *Brassica napus* variety reference collection on behalf of MAFF Plant Variety and Seeds Division. The reference collection comprises about 400 accessions of current and recent oilseed rape cultivars, including spring and winter types, that have been entered on to the UK National List. Hence these samples comprise the 'standard', should there be a query about the description or identity of a particular variety.
Contact: Bob Cooke.

Table 1. Cruciferous crops accessions in The Genetic Resources Unit, HRI, Wellesbourne, UK (Plus clubroot differentials and S-allele lines)

	Total	Available†	Safety-duplicated
Brassica carinata	309	6	309
Brassica juncea	89	57	46
Brassica napus - Portugal	55	55	55
Brassica napus biennis	83	64	71
Brassica napus napobrassica	220	195	168
Brassica napus oleifera	29	26	18
Brassica nigra	3	1	1
Brassica oleracea acephala	438	329	329
Brassica oleracea alboglabra	26	20	13
Brassica oleracea botrytis	950	525	351
Brassica oleracea capitata	1134	535	491
Brassica oleracea gemmifera	1005	384	216
Brassica oleracea gongylodes	46	37	23
Brassica oleracea italica	397	227	191
Brassica oleracea costata	82	74	72
Brassica rapa Broccoletto Gp	80	77	61
Brassica rapa Neep Greens Gp	2	2	0
Brassica rapa chinensis	40	36	23
Brassica rapa oleifera	5	4	4
Brassica rapa parachinensis	6	3	2
Brassica rapa pekinensis	63	38	8
Brassica rapa purpurea	1	1	1
Brassica rapa rapa	461	337	314
Brassica wild taxa	92	25	34
Raphanus caudatus	3	3	2
Raphanus maritimus	5	4	3
Raphanus raphanistrum	7	3	3
Raphanus sativus	699	397	314
Sinapis alba	20	16	7
Sinapis arvensis	3	0	0

† Availability determined by seed numbers and viability.

Source: Dave Astley, Genetic Resources Unit, HRI Wellesbourne.

Table 2. Brassica collections held at SASA†

UK Cabbage Cultivar Collection	663
UK Brussels Sprouts Cultivar Collection	221
UK Curly Kale Cultivar Collection	41
UK Turnip Cultivar Collection	235
UK Turnip Rape Cultivar Collection	74
UK Swede Cultivar Collection	176
UK Calabrese & Sprouting Broccoli Cultivar Collection	46
UK Radish Cultivar Collection	250

† UK Definitive Reference collections are also maintained by SASA for Distinctness, Unifomity and Stability tests.

Source: F.N.Green, Scottish Agricultural Science Agency, Herbage & Vegetable Crops Section, East Craigs.

Henry Doubleday Research Association, Ryton-on-Dunsmore

HDRA holds a collection of "heritage" varieties which are no longer available commercially. The collections are distributed to HDRA members and amateur gardeners, who also act as seed donors and multipliers. A majority of these accessions are duplicated at HRIGRU.
Contact: Bob Sherman.

John Innes Centre, Norwich (Brassica and Oilseeds Research Department)

The seed collection held by the Brassica and Oilseeds Research Department comprises around 1500 accessions (Table 3), including wild species, landraces, cultivars, breeding and research material. All the main *Brassica* species are represented, though not equally, together with a number of *Brassica* relatives such as *Sinapis*, *Crambe* and *Eruca*, and the more distantly related *Moricandia* and *Arabidopsis*. The primary purpose of the collection is to provide researchers within the Department, and (more rarely) at the Centre, with ready access to a range of material for use in research programmes, and as a short/medium-term storage facility for seed stocks produced during crossing programmes. In this sense it is very much a working collection, supporting the research programmes of the Brassica and Oilseeds Research Department and other departments at the John Innes Centre. There is no intention that it should function as a genetic resources genebank and no attempts are made to represent the full range of *Brassica* germplasm; for this we rely on the services provided by the recognised national and international genebanks. Users of the collection are free to make use of the seed stocks available (with permission of the owner of the seed) and to add any material coming in from their own research programmes or obtained from external sources. In addition, some individual researchers keep their own, private stocks of seeds.
Contacts: Eddie Arthur and Colin Morgan

Table 3. Seed collection of the Brassica and Oilseeds Research Department, JIC

Accessions			Total
Brassica juncea	accessions	280	
	breeding/research	83	363
Brassica napus	named accessions	50	
	unnamed accessions	30	
	breeding/research	251	331
Brassica nigra	all accessions		58
Brassica oleracea	named accessions	95	
	unnamed accessions	71	166
Brassica rapa	named accessions	46	
	unnamed accessions	100	146
Brassica carinata	named accessions	7	
	unnamed accessions	17	24
Sinapis alba	named accessions	30	
	unnamed accessions	64	
	research	222	316
Brassica species	number of accessions		48
	separate species	16	
Various oilseeds	species	25	
	cultivars	50	75

Total number of accessions	**1527**

Status of the national *Brassica* collections at the Nordic Gene Bank (NGB)

Gry Synnevåg
Landvik Research Center, Grimstad, Norway

Table 1 shows the *Brassica* collections in the Nordic Gene Bank, 1996. Currently, 366 accessions are stored in the NGB.

Table 1. Accessions of *Brassica* at NGB, November 1996

Brassica napus subsp. *oleifera* DC. (Rape)	45
Brassica napus var. *napobrassica*. (L.) Reichenb. (Swede)	59
Brassica napus subsp. *napus* L. (Forage Rape)	6
Brassica nigra (L.) Koch (Black Mustard)	1
Brassica oleracea var. *botrytis* L. (Cauliflower)	36
Brassica oleracea var. *capitata* f. *alba* (White cabbage)	84
Brassica oleracea var. *capitata* f. *rubra* (Red Cabbage)	11
Brassica oleracea var. *conica*	12
Brassica oleracea L. var. *botrytis* subvar. *cymosa* (Lam.) (Sprouting Brocco)	1
Brassica oleracea var. *gemmifera* DC. (Brussels Sprouts)	11
Brassica oleracea var. *gongylodes* L. (Kohlrabi)	1
Brassica oleracea var. *medullosa* Thell. (Marrow-stem Kale)	4
Brassica oleracea var. *sabauda* L. (Savoy Cabbage)	1
Brassica oleracea var. *sabellica* L. (Curlies)	11
Brassica rapa rapa subsp. *pekinensis* (Lour.) Rupr. (Pe-tsai)	2
Brassica rapa subsp. *oleifera* DC. (Turnip Rape)	44
Brassica rapa subsp. *rapa* L. (Turnip)	37
Total	**366**

New activities

Collection, regeneration and characterization

No systematic collecting of landraces nor varieties has been carried out during the last 2 years. Six accessions of Norwegian *Brassica napus* var. *napobrassica* and six accessions of *B. oleracea* var. *capitata* f. *alba* have been regenerated. The Norwegian and Danish *Brassica* material is characterized according to UPOV's guidelines.

Elaboration of a NGB Vegetable Catalogue

The vegetable catalogue includes the following *Brassica* species: *Brassica oleracea* var. *botrytis, B. o.* var. *conica, B. o.* var. *capitata f. rubra, B. o.* var. *capitata* f. *alba, B. napus* var. *napobrassica* and *B. rapa* subsp. *rapa*. For each of the species 8-10 important descriptors are selected and presented.

NGB project : "Criteria for selecting vegetable varieties for organic farming"

Head cabbage varieties stored in NGB were evaluated with regard to general requirements to yield and quality, but also with regard to:

- capacity to utilize organic fertiliers
- resistance against diseases and pests.

(Reference: Arenfalk, O., K. Henriksen and L. Hagelskjær. 1994 Evaluation of heading cabbage varieties for organic cultivation. Nordic Gene Bank- Publications. No 27. 31pp.)

Overview of commercial varieties on the official Norwegian variety list 1954-96.

The overview includes *Brassica napus* var. *napobrassica, B. napus* var. *napus, B. napus* subsp. *oleifera, B. rapa* subsp. *oleifera, B. rapa* subsp. *rapa* and *B. oleracea* var. *medullosa,* and will be incorporated in NGB's Nordic inventory database (NOIN).

A similar overview of *Brassica* varieties on the official Danish variety list 1970-96 is elaborated.

Regeneration and rationalization methods

Brassica regeneration procedures at the Nordic Gene Bank (NGB)

Gry Synnevåg
The Norwegian Crop Research Institute, Grimstad, Norway

The Nordic Gene Bank (NGB) has not elaborated specific guidelines for regeneration procedures in *Brassica*. The procedures mentioned in this paper are based on experience from *Brassica* breeding and seed production at The Norwegian Crop Research Institute, Apelsvoll Research Centre, division Landvik. The station is responsible for the regeneration of the Norwegian horticultural *Brassica* accessions stored in NGB.

The *Brassica* material stored in the Nordic Gene Bank includes:

- landraces orginating in a Nordic country
- varieties bred and marketed in a Nordic country
- varieties of non-Nordic origin extensively grown and adapted (older varieties) or entered in a "List of Varieties" in a Nordic country and which are not preserved by other genebanks
- varieties of non-Nordic origin with special characters valuable for Nordic cultivation and which are not preserved by other genebanks
- genetic stocks.

Most of the *Brassica* collection in NGB is classified as landraces and varieties bred and marketed in a Nordic country.

Regeneration is required for multiplication or rejuvenation. Multiplication is done when the quantity of seeds in the active collection for short- and medium-term conservation has dropped because of distribution, evaluation purposes and further utilization, to a level when restoring of seeds is needed. Multiplication is carried out if the seed sample comprises less than 10 000 seeds (preferably 20 000 seeds). Rejuvenation of seeds for long-term conservation in the base collection is done to maintain an acceptable viability percentage of the actual accession. Rejuvenation of the *Brassica* material is carried out if the germination rate is less than 75% (IPGRI standard <85%). The methods for multiplication and rejuvenation of *Brassica* accessions are, however, the same.

The species regenerated at the Landvik Research Centre are:

- white cabbage, early and late – *Brassica oleracea* var. *capitata* f. *alba*
- swede – *Brassica napus* var. *napobrassica* (L.) Reichenb.
- turnip – *Brassica rapa* subsp. *rapa* L.

The *Brassica* species are predominantly outbreeding species and populations are thus highly heterozygous and heterogeneous. The nature of these populations renders them particularly vulnerable to change in gene and genotype structure. Regeneration procedures should ensure that outbreeding is maximized as far as possible and selection forces minimized.

Common considerations in regenerating the different species

Seed production is carried out in polyethylene tunnels (4.5 x 24 m) to avoid contamination of foreign pollen. Pollinating insects (honey bees) are used to

obtain an effective random mating. To promote optimum growth conditions, the tunnels are provided with mechanisms for air circulation to obtain a good pollination, and to minimize attacks of different pests and diseases. The beehives should be placed at some distance from the flowering plants in the southwestern edge of the tunnel to obtain an active pollination.

To minimize genetic drift a minimum population size of 100 plants should be regenerated. Normally 150-200 plants are regenerated per accession.

Regeneration practices in the different species

Early white cabbage

Procedures for regenerating early cabbage are different from late cabbage because of limited storage capacity in early maturing types. Experiments with different methods of regenerating early cabbage have been carried out at the Landvik Research Centre (Vik 1989). On the basis of results of the experiments, seed production on winter sown transplants was recommended. The method can be summarized as follows:

- Sowing is carried out in 5-cm pots in December-January. Seedlings are placed in growth chambers at 18-20°C and 10 000 lux for germination. After germination the temperature is reduced to 15°C to obtain vigorous transplants.
- At a six-leaf stage (4-week-old transplants) plants are vernalized at 6°C (vernalizing temperature for early cabbage is 4-10°C), 12-h day, for 7 weeks.
- Planting of transplants in plastic tunnels is carried out in March-April. Distance between rows is 1 m; distance between plants is 40-50 cm.
- To obtain a good seed yield and uniform seed maturity, it is important that the soil fertility and humidity are high during the growing period. Drip irrigation is used to ensure constant humidity.
- Chemical treatment against pests and diseases (*Meligethes aeneus* and *Alternaria brassicae*) should be carried out before pollinating insects are placed in the tunnels.
- Seeds are harvested several times, first when 10% of the pods are ripe, and when the humidity is high (morning) to avoid seed shattering. In heterogeneous populations the problems of harvesting all plants with uniform ripeness can only be effectively met by harvesting individual plants separately.
- Gentle threshing of seeds is recommended to keep the seed quality (periphery drum speed: 10 m/sec, wide concave spacing).

By using this method a high seed yield and good seed quality have been obtained (95% germination capacity).

Late white cabbage

To avoid genetic drift regeneration is carried out in regions where the accessions are adapted. Landraces from northern Norway adapted to long day and low temperatures during the growing season, are if possible grown in their original area, and at least 100 heads are chosen randomly for further seed production. Seed production is carried out at the Landvik Research Centre.

Different methods for seed production in late cabbage are recommended in the literature. Most methods are 'seed-head-seed' methods, where seed production is

based on stored roots with or without heads treated in different manners. 'Seed to seed methods' are also developed but less used. Experiments with different methods of seed production of late cabbage also have been carried out at the Landvik Research Centre (Jonassen 1971).

Roots with pruned heads are treated against storage diseases and overwintered at 0°C. If roots are stored with intact heads a storage temperature of 2°C and additional light are recommended to obtain maximum seed yield. Planting in plastic tunnels is carried out in April-May. Planting distances are 1 m between rows and 60 cm between plants. The subsequent procedure is similar to early cabbage.

Swedes and turnips

Experiments were carried out at the Landvik Research Centre with different commercial field methods for seed production of swedes and experiments with seed production of swedes and turnips in plastic tunnels (Jonassen 1970).

Regeneration of swedes and turnips for genebank purposes is carried out in plastic tunnels. Landraces from northern parts of Norway are grown in their original habitat, and seed is regenerated at the Landvik Research Centre from stored roots. In some cases seed to seed methods are used. Normally 150-200 plants per accession are regenerated.

Seed production based on stored roots

- Experiments with distances between rows (30, 45 and 60 cm) and between plants (7.5 and 15 cm) did not show significant differences in total seed yield per area. However, increased distance between rows and plants gave a higher seed yield per plant. Normally 60 cm between rows and 30 cm between plants are recommended. To minimize competition between plants, wide spacing is preferred for genebank purposes, thereby also maximizing seed output per plant. This is particularly important when small numbers of plants are being grown.
- Experiments with different amounts of N fertilizer show that swedes give higher returns for high level of N than turnip. An amount of 20 kg N is recommended.

Seed to seed production

- Plant raising as for early cabbage.
- Vernalization, 5-7°C, 6 weeks, before planting in plastic tunnels.
- Distance between rows is 60 cm; between plants is 30 cm.

Experiments show great variation between different varieties in their resistance to flower induction. Norwegian landraces have shown a greater resistance than Danish swede varieties (Johannesen 1976). The root to seed method may therefore be recommended to avoid changes in the genetic material.

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Current research on the distribution of genetic diversity in Dutch white cabbages

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Introduction

The historical background of the cabbages and Brussels sprouts originating in the Netherlands made it possible to reduce the number of accessions that needed to be included in the CGN collection. Based on passport data, morphological inspection and the expertise of breeders and researchers, it was possible to determine which material could be bulked into the actual genebank accession. This process was validated using isozyme markers (Hintum *et al.* 1996).

During the isozyme analysis the question arose how far one could go, on genetic grounds, with bulking accessions. The hypothesis that, for example, all Dutch white cabbages were merely different samples from the same genepool, could not be rejected on the basis of the isozyme patterns. Therefore an experiment was set up to determine genetic diversity in Dutch white cabbage accessions based on molecular markers. The project, which has started in May 1996 and will run for 2 years, will give answers to questions such as:

- How large is the genetic diversity within white cabbage accessions, and how do cultivars compare to landraces?
- How is the genetic diversity distributed over white cabbage accessions, and how should a core collection be compiled?
- How should duplicates be defined, how frequent are they and how can they be traced?
- How frequent do rare alleles occur, and do they occur in high frequencies in very few accessions, or in low frequencies in relatively many accessions?
- On the basis of the current procedures for multiplication, can genetic erosion as a result of genetic drift be expected, and if yes, how could this be prevented?
- Only considering the conservation of alleles, could accessions be bulked without increasing the risk of genetic erosion?

Material and methods

To determine the differences between similar accessions, and to put these differences in the context of the entire diversity of the genepool, 51 accessions were selected. These consisted of the following groups:

- all accessions from the 'Brunswijker', 'Gouden Akker', 'Roem van Enkhuizen', 'Delikatesse', 'Amager Kortstronk' and 'Herfstdeen' duplication groups,
- most accessions of the duplication group 'Langedijker',
- an additional cross-section of the Dutch white cabbage genepool, and
- an additional cross-section of the total white cabbage genepool.

Care was taken that all white cabbage accessions included in the previous electrophoretic screening were included in the selected material.

Two types of molecular markers will be used: AFLPs and microsatellites. For the AFLP analysis four primers will be used, yielding about 200 data points per plant of which a substantial part can be expected to be polymorphic. For the microsatellite analysis six sets of three primer pairs each will be used.

Per accession a minimum of 30 plants will be screened. For the codominant markers this means that there is a minimum probability of 95% of detecting an allele which occurs with a frequency of 5%, for a dominant marker there is a minimum probability of 95% of detecting a phenotype which occurs with a frequency of 10%.

Reference

van Hintum, Th.J.L., I.W. Boukema and D.L. Visser. 1996. Reduction of duplication in a *Brassica oleracea* germplasm collection. Genetic Resources and Crop Evolution 43:343-349.

Brassica Core Collection

Brassica oleracea Core Collection

Dave Astley[1], Ietje Boukema[2] and Theo van Hintum[2]

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[2] Centre for Genetic Resources The Netherlands (CGN), Wageningen, The Netherlands

A *Brassica oleracea* core collection (BOCC) was developed to support pest and disease resistance screening research in the EU AIR3 project: 'The location and exploitation of genes for pest and disease resistance in European genebank collections of horticultural brassicas' (Boukema *et al.* 1996). The project is a collaboration between Horticulture Research International (HRI), the Centre for Genetic Resources The Netherlands (CGN) and the Instituto Superior de Agronomia (ISA), Lisbon. The objectives were:

- to screen the cultivated *Brassica oleracea* genepool for resistance to downy mildew (*Peronospora parasitica*), white blister (*Albugo candida*) and the cabbage aphid (*Brevicoryne brassicae*);
- to study any resistances identified;
- to publicize the research, the BOCC and associated project data;
- to make the BOCC available to other researchers depending upon seed availability.

The research teams developed their screen protocols, which in turn defined the number and quality of accessions required from the genetic resources collections. CGN and the Genetic Resources Unit of HRI (HRIGRU) identified accessions of *B. oleracea* in their collections representing the range of crop type/form and ecogeographical location as a preliminary core collection.

Subsequently the ECP/GR *Brassica* database (Bras-EDB) at CGN was used to identify material available in other genetic resources collections which was not represented in the core. The *B. oleracea* accessions in the Bras-EDB were subdivided hierarchically using botanical and provenance data by the BOCC path indicator designed by Theo van Hintum at CGN (Boukema *et al.* 1996). Material was identified in this structured genepool which was not represented in the HRIGRU/CGN preliminary core collection. Subsequently seed was obtained from other genebanks. Significant collections were received from ISA Lisbon, IPK Gatersleben, Germany and INRA Le Rheu, France. The project team were keen to fill gaps for specific crops such as collards from the USA; or material from specific geographic areas, especially the east European Republics, Spain and Turkey. A majority of the accessions received required regeneration prior to their addition to the project core collection or use in the research project. Accessions in the project core collection were tagged BOCC as an identifier in the Bras-EDB. Preliminary data produced by the research will be stored in a database file linked to the Bras-EDB by the BOCC tag, maintained at CGN and HRIGRU. The project 'core collection' was developed solely for use within the project research, but its development did provide valuable insights into the philosophy and practicalities of the core collection concept.

The collection used for this project has proved extremely valuable in identifying 'hot spots' for resistance in the *B. oleracea* genepool and its geographic distribution.

However, the project collection does not meet several of the criteria for Brown's (1989) definition of a core collection: it represents a number of collections rather than a single collection; the size and content was fixed rather than fluid; the content was biased for certain taxonomic and ecogeographic groups. The project research teams had a significant influence on the size, structure and fixed nature of the project collection. The collection size was determined effectively on what was achievable using the three research protocols within the time of the project. The three teams agreed to screen the same accessions in the same order, thus once scored an accession was fixed in the collection. User preconceptions on the value of material from parts of the genepool or ecogeographic areas, based on experience and the scientific literature, led to a biased collection.

Other research workers have shown an interest in using the BOCC. But it is clear, and with hindsight understandable, that individuals have their own research objectives, interests and constraints. Therefore discussions on the use of the BOCC by other scientists generally have been directed towards manipulating its content to suit specific needs.

The EU project work has highlighted for the genebanks involved the practical problems involved in the development and maintenance of a relatively simple project 'core collection'. The main problems were:

- the acquisition of material;
- receipt of small quantities of seed from other donor genebanks;
- the research requirement for large amounts of high quality seed, inevitably this meant regeneration to meet immediate and future demands.

However, the project did prove that it is possible to coordinate three genebanks efficiently in support of a specific objective. It remains to be seen if all the genebanks represented in the Bras-EDB could play an active role in the conservation, regeneration, documentation and distribution of their collections in the coordinated development and executive maintenance of a *Brassica oleracea* core collection.

Our experience leads us to conclude that the most efficient use of genetic resources collections will be through constructive dialogue between genebank managers and potential users. Brown viewed a core collection as being fluid because for each collection, the content and associated data inevitably change through time. Each new user will not only look at the genepool from a different perspective, but potentially will be looking at a 'different genepool' to the last user. The situation is far more complex when more than one collection is considered. Therefore two vital components have to be available to support the manager / user dialogue:

- a central crop database;
- a mechanism for analyzing these data, such as the BOCC path indicator.

If these components are available, the genebank manager or central crop database manager can consider the possible design for a 'core collection' to suit each request based on the criteria supplied by the user and perhaps the more pragmatic considerations of availability, etc.

Conclusions

1. The BOCC is not a core collection as defined by Brown (1989).
2. The development of the BOCC by the EU project demonstrated the value of a central crop database, such as the Bras-EDB, in providing potential users with

access to structured information relating to a number of germplasm collections.

3. For the EU project, the choice of material and the interpretation of the results were simplified by the application of the BOCC path indicator to the Bras-EDB data which structured the data in an hierarchical format.
4. The project researchers had a very significant influence on the selection criteria and the size and structure of the BOCC collection to be used.
5. Users of a subset of accessions want that collection to be identifiable, e.g. BOCC, and any associated data produced to be maintained with a link to the accession database. This places the onus on genebank managers to collect and maintain all the meta-data plus relevant primary data linked to the collection provenance, research protocols and publications.
6. The success of such work depends on all national programmes being willing and / or able to provide material.
7. The major problem associated with a core collection based on a network of collections is the increased workload for the central crop database manager, such as acting as a clearing house for requests, analysis the central database and coordination of seed distribution from a number of national programmes, many of whom have multiple crop responsibilities.

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In situ conservation

Monitoring of (2*n*=18) wild *Brassica* populations in Italy and Croatia

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Introduction

The wild species of the *Brassica oleracea* L. group (2*n*=18) have been a subject of interest and investigation both recently as well as in the past. One reason for this interest can be traced to their importance as close allies of the cultivated forms of *B. oleracea*. They all share the same genomic constitution and belong to its primary genepool, and all can interbreed with *B. oleracea* to various extents of fertility, and so provide a potential source of genes that can be used in plant breeding programmes.

Ecogeographic knowledge is fundamental to genetic conservation and use. This kind of information is essential for the planning of effective conservation strategies and can provide guidelines for choosing potential breeding material, on the basis of ecological and environmental indications.

The distribution of Mediterannean (2*n*=18) brassicas has been extensively investigated in the last 15 years (Snogerup *et al.* 1990). However, areas for which there is little ecogeographic knowledge are still present, for example, the Dalmatian islands in Croatia. Furthermore, regular monitoring of all the populations is desirable in general. This is particularly true for Sicily, where the high genetic variability displayed suggests the existence of a centre of diversity for this group of brassicas (Gustafsson 1995).

During the summer of 1996, as part of our theses for a Master of Science in the Conservation and Utilization of Plant Genetic Resources (School of Biological Sciences, University of Birmingham) the authors organized two parallel expeditions with the objective of monitoring (2*n*=18) wild *Brassica* sites in southern Italy and in Croatia (Eastwood 1996; Maggioni 1996). Voucher herbarium samples and seeds also were collected in various amounts for ecogeographic and taxonomic studies.

Monitoring in Italy

The aim of the expedition to southern Italy was to visit as many (2*n*=18) wild *Brassica* population sites as possible, in order to collect a wide range of samples of four Sicilian taxa: *B. macrocarpa* (Guss.) Caruel, *B. villosa* Biv., *B. rupestris* Rafin. and *B. incana* Ten. The main objective was to collect herbarium specimens for the scoring of characters for a taxonomic numerical analysis.

For this purpose, mainly sites already clearly located were visited. However, the inspection of a couple of sites of dubious status was included among the objectives of the research. This included a thorough survey of the island of Levanzo (Egadi islands, Sicily) where *B. macrocarpa* had been originally found by Gussone (1828). Di Martino and Trapani (1965) were unable to find *B. macrocarpa*

in their survey of the island although its presence was not ruled out. The second dubious site which was investigated was on Tremiti islands, Puglia, where, according to Snogerup *et al.* (1990), the status of *B. incana* was not known.

Monitoring of the Egadi islands (Sicily)

The Egadi islands are a group of islands off the west coast of mainland Sicily. *Brassica macrocarpa* is an endemic species to this archipelago. Its distribution on the islands of Favignana and Marettimo was assessed thoroughly. Table 1 gives a summary of the monitoring activities which have occurred during the last century on the three main islands of Favignana, Marettimo and Levanzo. Data from 1996 are the result of the present study. The other dates refer to botanic and ecogeographic expeditions carried out respectively by Lojacono Pojero (1889), Francini and Messeri (1935 through 1947), Di Martino and Trapani (1965), Snogerup and Gustafsson (1973 and 1984).

The monitoring undertaken in 1996 showed that the Favignana populations are thriving well. There was noticeable regeneration in all populations. No evident signs of threat were observed, although occasional grazing at the base of the cliffs occurred. The apparent decline in population size in the last 10 years may be due to the difficulties associated with estimating the number of plants growing on inaccessible cliffs. However, more rigorous monitoring of these populations is recommended given the uniqueness and importance of these populations.

On the island of Marettimo the existence of more populations than previously indicated was verified. The general impression was of well thriving and unthreatened populations in a similarly very well preserved environment.

A thorough survey by foot and boat was carried out on the island of Levanzo. Special attention was given to the observation of the inaccessible cliffs of the northeast side of the island, to which Di Martino and Trapani (1965) confined their last hopes of finding any remaining *B. macrocarpa*. The author can confidently conclude that this species is not present on the island any more. A history of intense farming on this island, which was confirmed by local people, may be one of the reasons for its recent disappearance. Being the smallest of the three islands, Levanzo is more susceptible to human disturbance and provides less suitable habitat for *Brassica*.

Monitoring in mainland Sicily

The *Brassica* populations in Sicily appeared to be more threatened than on the Egadi islands. Intense levels of grazing and signs of recent fires were observed within some sites or in close proximity. Uncontrolled fires were commonly spotted in many places on the island. Quarrying activity in the northwestern part of the island is high and was observed among *Brassica* populations. Several populations were limited to only a few plants. A decline in size was noticed for *B. villosa* subsp. *villosa* at the type site for *B. villosa* at Monte Occhio (Palermo). The population of *B. rupestris* just below the village of Isnello (Palermo) is also very reduced in size. Gustafsson *et al.* reported in 1984 that the population of *B. rupestris* at Capo Gallo (Palermo) was threatened by grazing and had been reduced to only 80 plants (quoted in Snogerup *et al.* 1990). There was no sign of this population in the proximity of the lighthouse, although the author cannot account for the possible presence of some plants in other inaccessible areas.

It is considered possible that many undiscovered populations do exist on the island, since there are plenty of areas with suitable habitats. Extensive surveying would be as valuable as difficult and time consuming. Recommendations for higher protection and monitoring of the Sicilian populations, as suggested by Gass

et al. (1995) are advisable. Table 2 summarizes some of the results of monitoring in Sicily.

Table 1. Monitoring *Brassica macrocarpa* at Egadi islands

	1889	1935-47	1965	1973-84	1996
Favignana	Y	Y	Y		
Montagna Grossa - East				6000/2200	2400/1100
Montagna Grossa - West				>1000	>1000
Marettimo	Y	Y	Y	Y	Y
Punta Bassana		Y		150/65	360/235
Monte Falcone		Y		Y	30/19
Pizzo Campana		Y			Y
Libàno		Y			Y
Punta Lisandro					Y
Levanzo	Y	N	N?	?	N

Y -= found; N = not found; N? = doubtful presence.
Ratio = Total no. of plants/no. of reproductive plants.

Table 2. Monitoring in mainland Sicily

Capo Gallo	*B. rupestris*	1984: 80 plants; 1996: 0 plants
Isnello	*B. rupestris*	presence of hybrids with cultivated brassicas
Scurati	*B. villosa*	quarrying activity within the site
Monte Occhio	*B. villosa*	very limited population
Acqudolci	*B. incana*	new site at "Contrada Imperia"
Torrenova	*B. incana*	new site along SS 113

Monitoring on the Tremiti islands (Puglia)

The Tremiti islands are a group of islands off the coast of Puglia, in the Adriatic Sea. *Brassica incana* was collected here in 1906, but the status of this population has not been verified since then (Snogerup *et al.* 1990). A thriving population of at least 1500 plants (about 310 reproductive) was found in the southern part of the island of S. Domino; about 1200 plants are growing on gentle slopes under pine trees and in open grassy areas, where domestic herbivores are not allowed to graze freely. The other 300 plants were observed on the inaccessible vertical cliffs.

Monitoring along the Dalmatian coast (Croatia)

The distribution, ecology and status of *Brassica incana* in Croatia is largely based on ecogeographic data extracted from herbarium specimens collected in the 19th and early 20th centuries. In the last 15 years there have been numerous IBPGR-sponsored collecting missions for ($2n$=18) Mediterranean brassicas. This has resulted in a better understanding of the ecogeography and taxonomy of Mediterranean brassicas. Unfortunately these collecting missions have not included Croatia in their remit. This has probably been due to the political instability caused by a 5-year civil war. The last known collecting of *Brassica incana* from the Dalmatian coast dates back to those made on the islets of Obljak and Kosor (off the SW coast of Korcula) in 1974 by Snogerup and Engstrand. The original collections are based on seed sampled from only four individuals. They are currently conserved at the Institute of Systematic Botany, University of Lund, Sweden.

The main objectives of the survey mission in Croatia were:

- to assess the conservation status of *Brassica incana* on the Dalmatian coast

- to collect a representative sample of seed for *ex situ* conservation
- to collect herbarium specimens for a taxonomic study.

The comprehensive ecogeographic data collected would assist in making recommendations for conservation and setting priorities for future action.

Table 3 lists all the known and dubious localities of *B. incana* along the Dalmatian coast with a summary of the results of this survey. The localities are based on a literature review and interviews with Croatian botanists (Snogerup *et al.* 1990; Trinajstic and Dubravec 1986; Rac and Lovric 1991; Lovric, pers. comm., 1996; Kamenjarin, pers. comm. 1996).

In Croatia a number of endemic variants of *B. incana* have been recognized by several authors, which has resulted in a large number of synonyms (Visiani 1850, 1852; Ginzberger and Teyber 1921; Trinajstic and Dubravec 1986). The described species include *Brassica incana* Ten., *Brassica botterii* Vis., *B. cazzae* Ginzberger & Teyber and *B. mollis* Vis. The classifications are largely based on differences in siliqua characters.

Table 4 is a summary of the populations of *Brassica incana* encountered during the survey mission and details of seed collected from those populations. If we consider the classification of *Brassica* by Trinajstic and Dubravec (1986) the six populations would represent two distinct taxa. Populations EAST01-EAST04 would be classified as *B. botterii* subsp. *mollis* (Vis.) Trinajstic, *comb. nov*. and EAST05-EAST06 as *B. incana* subsp. *incana*.

Populations EAST01-EAST04 occurred on small, rocky islets off the SW coast of the island Korcula. Estimated population sizes varied from 15 to 300 individuals. *Brassica incana* (EAST01-EAST04) grows in crevices among rocks in a zone starting at 5-10 m asl and ending at the beginning of the maquis vegetation; it was absent in areas where there was tall, thick maquis.

The two populations of *B. incana* (EAST05-EAST06) found in Komiza Bay, on the island of Vis, occurred on vertical, dolomite cliffs approximately 300 m asl. Individuals were observed growing on crevices and ledges of the cliff face as well as at the base of the cliff. Individuals of EAST05 were observed growing on the limestone walls of abandoned terraces at the base of the cliff. Estimation of population sizes was difficult to establish owing to the inaccessibility of the cliffs.

The timing of the survey mission coincided with the beginning of collecting window for *B. incana.* Seed was collected from all the populations visited. Details of the collections are given in Table 4. The habitat preference of *B. incana* (particularly EAST05-EAST06) made the sampling and collection of seed very difficult. One of the objectives of the survey mission was to collect seed for long-term *ex situ* conservation. Croatia does not currently have a national genebank for germplasm conservation or a policy for germplasm exchange. The collected seed was therefore deposited at the University Botanical Garden, Zagreb, where it will be used in taxonomic research. Negotiations are currently underway to obtain a representative sample of the seed so that it can be conserved at the designated *Brassica* genebank in Madrid.

A survey of the islands comprising the Elaphite archipelago near Dubrovnik by boat and foot (Lopud)did not locate any *Brassica* populations. Similarly, a survey of Svetac by boat did not locate any *Brassica* populations. However, the surveys of these islands were only conducted superficially because of the difficulties associated with access to seaward cliff faces by boat and bad weather conditions.

Unfortunately the islands of Velika and Mala Palagruza (situated in the middle of the Adriatic sea) and Susac were not surveyed. These islands are very remote

and require special arrangements in order to visit them. However, recent reports suggest that there is a large population of *B. incana* on Velika Palagruza (Kamenjarin, pers. comm., 1996).

Table 3. A summary of the known and dubious localities for *B. incana* in Croatia with the results of this survey

Localities	Survey status	Present/Not present
Known		
Island of Vis (Komiza Bay)	Surveyed	P (2 populations)
Island of Svetac	Surveyed	NP
Island of Susac	Not surveyed	–
Island of Kamen	Not surveyed	–
Korcula		
Islet of Obljak	Surveyed	P
Islet of Kosor	Surveyed	P (2 populations)
Palagruza		
Velika Palagruza	Not surveyed	–
Mala Palagruza	Not surveyed	–
Unknown or dubious		
Korcula		
Islet of Stupa	Surveyed	P
Elaphite archipelago		
Šipan	Surveyed	NP
Jaklan	Surveyed	NP
Mišnjak	Surveyed	NP
Ruda	Surveyed	NP
Lopud	Surveyed	NP
NE Biševo	Surveyed	NP

P = Present, NP = Not Present.

Table 4. Monitoring in the Dalmatian coast

Coll. no.	Location	Pop. size	Plants sampled	No. of seeds collected
EAST 01	Obliak	300	30	1200
EAST 02	Kosor	15	5	100
EAST 03	Kosor	30	7	200
EAST 04	Stupa	50	4	100
EAST 05	Vis	200	5	250
EAST 06	Vis	150	4	100

Assessment of conservation status

Owing to the remoteness of the locations for *B. incana* in Croatia and the habitat preference of populations, there are no imminent threats from human disturbance. Possible threats to individual populations include reduced fitness due to small population size and encroachment of *Brassica* habitat from maquis scrub (*Pistacia lentiscus*) leading to interspecific competition. Population EAST02 was comprised of only 15 individuals. The siliqua and seeds of this population were small and poorly developed. Observed regeneration was poor.

In a recently published check list of Croatian flora, Plazibat (1996), having adopted the classical taxonomic nomenclature, has assigned the IUCN Red List Category of Rare to *B. incana* and Endangered to *B. botterii*, *B. cazzae* and *B. mollis*. The latter three species are considered to be endemic to Croatia.

Whichever taxonomic classification is applied for *Brassica* in Dalmatia, whether they are treated as individual species, subspecies or ecotypes of *B. incana*, the taxa

should be considered as Vulnerable (IUCN 1994) in Croatia. This is based on their limited distribution in Croatia and the small, isolated populations.

Recommendations for conservation and research

A concerted effort to obtain a sample of the seed collected by the survey mission for *ex situ* conservation should be initiated. This could be steered by the ECP/GR *Brassica* Working Group. A future collecting and survey mission should be planned for the near future. The islands not visited during 1996, for example Susac, Kamen and Palagruza, should be surveyed and seed collected for *ex situ* conservation. The participation of a local counterpart, such as Juraj Kamenjarjn at the University of Split is also necessary to confirm the presence or absence of *Brassica* populations with more certainty. Financial and logistical support would be required from an international organisation such as IPGRI.

The level and distribution of genetic diversity within and between the *Brassica* populations in Croatia should be investigated using biochemical and/or molecular techniques such as isozyme and RAPD analysis (Hurtrez-Boussès *et al.* 1996, Lannér-Herrera 1996). Comparisons with populations of *B. incana* in Italy could then be made. This could also help to elucidate some of the taxonomic problems and highlight those populations which are most genetically distinct.

Genetic diversity

Sicily is considered to be a centre of diversity for (2*n*=18) Mediterranean brassicas owing to the high level of inter- and intraspecies diversity there. The character 'leaf hairiness' in *B. rupestris* is often quoted as a sign of intraspecific variability within this species. A large amount of variation in hair type has been shown for *B. rupestris*, particularly for the cliff system of Rocca Busambra, Palermo (Gómez-Campo and Gustafsson 1991). The plants scored in this study also showed great variability regarding this character, both between and within populations. The five specimens collected at Rocca Busambra, above 'Bosco della Ficuzza', showed four types of leaf hairiness. They were collected within a few square meters (see Table 5). The complexity of the genetic inheritance of leaf trichomes density and pattern has been described by Eastwood (1996) for *B. incana*. There is potential for utilizing leaf hairiness to confer insect resistance in nonvegetable crops such as oilseed rape (*Brassica napus* L.).

The number of seeds in the beak is another interesting trait due to its evolutionary implications (Gómez-Campo and Gustafsson 1991). The examination of the specimens collected during the present expedition offered some interesting results regarding the unusual presence of plants with 1-seeded or 2-seeded beaks. The sites where such plants were identified are listed in Table 6.

Table 5. Variability of *B. rupestris* at Rocca Busambra

Accession	Leaf hairiness
MAG 47	Sparse hairs
MAG 48	Sparse hairs
MAG 49	Glabrous
MAG 50	Glabrous, except for sparse hairs at the margin
MAG 54	Hairs (<20/mm^2) regularly distributed only on top leaf

Table 6. Populations of wild brassicas with individuals bearing seeds in the beak

Species	1-seeded	2-seeded
B. villosa	Pizzolungo	
B. rupestris	Monte Pellegrino	

B. incana	Scafa, S. Domino, Komize	Scafa, Acquedolci

The recognition of endemic variants of *B. incana* in Croatia by several authors on the basis of siliqua characters suggests the presence of between-population variation. The majority of the populations are isolated from each other spatially, either by sea or by rocky escarpments. Strong genetic differentiation between populations has been shown for *B. insularis* Moris on Corsica using allozyme analysis (Hurtrez-Boussès *et al.* 1996). The results are consistent with the effects of genetic drift and reduced geneflow between isolated populations.

Conclusion

The value of wild brassicas is shown today by the ongoing effort in research laboratories to transfer insect resistance genes and anti-cancer properties from wild brassicas into crops (Vines 1996).

These plants are precious genetic resources endemic to the Mediterranean area. By signing the Convention on Biological Diversity in Rio, Brazil, in 1992 and the Declaration of Leipzig, Germany, in 1996, European countries have accepted the principle and the responsibility of a sustainable conservation and use of biological resources.

A serious commitment for the conservation of this group of brassicas should consequently be expected from the respective countries' decision-makers. Conservation should not only mean survival of the species, but also knowledge of the distribution, ecology and genetic diversity of the existing populations. On the basis of this sounded knowledge it should be possible to develop and implement an integral management plan for the conservation and use of wild brassicas.

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Report on activities and planning for *in situ* and *ex situ* conservation in Sicily[14]

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The germplasm bank of the Palermo Botanical Garden keeps 25 accessions of *Brassica* collected in Sicily and in the surrounding islands (Table 1). The interest for this group of species is devoted to the localization of new sites of origin, the study of the population biology and the evaluation of the seed quality during the conservation. The investigation of the ecotypes has been carried out by comparative analyses regarding the behaviour of the species in both their natural habitats and in the Botanical Garden under controlled ecological conditions. Research activity is devoted also to the morpho-anatomical characterization of the *Brassica* genotypes as well as to the finding of the best conditions of *ex situ* conservation by cyto-physiological evaluations. The bank is available to receive duplicates by other institutions.

Table 1. *Brassica* accessions maintained in the genebank of the Palermo Botanical Garden

Species	Origin	Year	No.
Brassica amplexicaulis (Desf.) Pomel subsp. *souliei* (Batt.) Maire et Weiller	Alimena (Palermo)	1993[†]	1
Brassica fruticulosa Cyr.	Cefalù (Palermo)	1993	1
Brassica macrocarpa Guss.	Favignana Island (Trapani)	1994	1
Brassica nigra (L.) Koch	Valderice (Trapani)	1994	1
Brassica rupestris Rafin. subsp. *rupestris*	Rocca Busambra (Palermo)	1994	1
	Rocca Busambra (Palermo)	1995	1
	Rocca Busambra (Palermo)	1996	1
Brassica rupestris Rafin. subsp. *hispida* Raimondo et Mazzola	Mt Pizzuta (Palermo)	1995	2
Brassica villosa Biv. subsp. *bivoniana*	Mt Inici (Trapani)	1994[‡]	1
(Mazzola et Raimondo) Raimondo	Mt Inici (Trapani)	1995[‡]	1
et Mazzola	Mt Inici (Trapani)	1996	1
	Zingaro (Trapani)	1996	1
	Caltabellotta (Agrigento)	1996	1
Brassica villosa Biv. subsp. *drepanensis*	Mt San Giuliano (Trapani)	1994[§]	1
(Caruel) Raimondo et Mazzola	Mt San Giuliano (Trapani)	1995[§]	1
	Mt San Giuliano (Trapani)	1996	4
Brassica villosa Biv. subsp. *glabrata*	Isulidda (Trapani)	1994[¶]	1
Raimondo et Mazzola	Isulidda (Trapani)	1996	4

[†] subsp. *B. souliei* (Batt.) Batt.

[‡] subsp. *B. bivoniana* Mazzola et Raimondo.

[§] subsp. *B. drepanensis* (Caruel) Damanti.

[¶] subsp. *B. rupestris* Rafin.

[14] Update May 1997.

As regards the recent activities and planning for *in situ* conservation in Sicily, action was taken to target populations located in sites already existing in natural reserves. This was done in particular for:

- the island of Marettimo to protect populations of *B. macrocarpa*
- the Zingaro reserve in the Trapani district to protect populations of *B. villosa* subsp. *drepanensis* and *B. v*. subsp. *bivoniana*
- the Madonie Park in the Palermo district to protect populations of *B. rupestris* subsp. *rupestris*
- the Pellegrino Mountain in the Palermo district to protect populations of *B. rupestris* subsp. *rupestris*
- the natural Park Nebrodi in the Messina district to protect populations of *B. incana.*

In the near future, the following actions will be taken by the Palermo Botanical Department:

- to contact the local administrations and to discuss the possibility of increasing the grazing along the foothills in order to reduce the vegetative production and eliminate fires;
- to plan the monitoring of the demographic structure of the populations;
- to stimulate further characterization of the infraspecific taxa, e.g. by applying molecular marker systems and seed physiology.

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Appendix II. Abbreviations and acronyms used in the text

AARI	Aegean Agricultural Research Institute, Menemen, Izmir, Turkey
AIR	Agriculture and Agro-Industry Research Programme
BAZ	Bundesanstalt für Züchtungsforschung an Kulturpflanzen (Federal Centre for Breeding Research on Cultivated Plants), Quedlinburg, Germany
BOCC	*Brassica oleracea* Core Collection
BPGV	Banco Português de Germoplasma Vegetal, Portugal
Bras-EDB	European Database for *Brassica*
CGN	Centre for Genetic Resources The Netherlands, Wageningen, The Netherlands
CNR	Consiglio Nazionale delle Ricerche (National Research Council), Bari, Italy
CRF	Centro de Recursos Fitogenéticos, Madrid, Spain
EC	European Commission
ECP/GR	European Cooperative Programme for Crop Genetic Resources Networks
EGDS	Eastern European Germplasm Documentation Systems Project
EU	European Union
HRI	Horticulture Research International, Wellesbourne, UK
IGER	Institute for Grassland and Environmental Research, Aberystwyth, UK
IHAR	Plant Breeding and Acclimatization Institute, Radzikow, Poland
IIPGR	Institute of Introduction and Plant Genetic Resources, Sadovo, Bulgaria
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany
ISA	Instituto Superior de Agronomia, Lisbon, Portugal
ISHS	International Society for Horticultural Science
MBG	Mision Biologíca de Galicia, Pontevedra, Spain
NGB	Nordic Gene Bank, Alnarp, Sweden
NVRS/IVT	National Vegetable Research Station, Wellesbourne, UK / Instituut voor Tuinbouwplantenveredeling, Wageningen, The Netherlands
RICP	Research Institute of Crop Production, Prague, Czech Republic
RICP	Research Institute of Crop Production, Piestany, Slovakia
SIA	Servicio de Investigaciónes Agrarias, Spain
UPM	Universidad Politécnica de Madrid, Spain
UPV	Universidad Politécnica de Valencia, Spain