European Cooperative Programme for Crop Genetic Resources Networks

ECP/GR

Report of a Working Group on *Brassica*

IPGRI

Extraordinary meeting, held jointly with the Third Coordination Meeting of the GEN RES CT99 109-112, 8 –9 February 2002, Vila Real, Portugal

L. Maggioni, G. Thomas and E. Lipman, *compilers*

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PART I. DISCUSSION AND RECOMMENDATIONS

Introduction

Opening of the meeting

Eduardo Rosa, Portuguese member and Vice-Chair of the Working Group on *Brassica*, opened the meeting with an introductory welcome, also on behalf of the University of Trás-os-Montes and Alto Douro (UTAD).

Chairman's introduction

Grégoire Thomas, Chair of the Working Group on *Brassica*, addressed the participants with the following introductory welcome:

"We have already met this morning for this joint meeting giving information on the GEN RES project. Now, we are opening the *Brassica* Working Group meeting in Vila Real, first by thanking UTAD and E. Rosa for their organization and hospitality. Thanks Ed for your friendly help! The *Brassica* Working Group is an "old" ECP/GR Working Group! The previous meeting was held in Rome in 1996 during Phase V. The beginning of ECP/GR Phase VI introduced new options, especially the idea of assembling Working Groups in Networks. The *Brassica* Working Group, although it also deals with non-vegetable species, was included in the Vegetables Network, for which I act as facilitator. This Network Coordinating Group, including Chairpersons and Vice-Chairpersons of the existing Working Groups on *Allium*, *Brassica* and Umbellifer crops, met here in Vila Real in 2000, together with experts of other vegetable genetic resources (Solanaceous species, cucurbits, leafy vegetables and minor vegetable crops). At this meeting, a workplan was proposed to the ECP/GR Steering Committee, including the following items:

- focus on activities that are common to different Working Groups;
- proposal for the establishment of new Working Groups (a Solanaceae Working Group has subsequently been approved);
- proposal to hold Working Group meetings together with the respective GEN RES projects in order to facilitate links and to limit the costs of meetings, by dedicating ECP/GR funds to the participation of national representatives not belonging to the GEN RES projects. It is thanks to this that we have the opportunity to meet here.

As we have not met recently, we have a big job in front of us, including reviews of what has been done recently in our countries and what should be planned. This is why Lorenzo and I have decided to concentrate reporting on specific subjects, rather than having country reports.

I would like to finish this introduction by saying that we have to discuss each of the different tasks, and please propose at the end of each session concrete proposals for future work. I hope we shall do that on a free and informal discussion basis, but with effective proposals."

ECP/GR briefing and outcome of the Mid-term Steering Committee meeting

Lorenzo Maggioni, Coordinator of the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR), welcomed the participants to the extraordinary meeting of the Working Group on *Brassica*, jointly organized with the third meeting of GEN RES project CT99 109-112 on "*Brassica* collections for broadening agricultural use, including characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop". He said that ECP/GR supported the participation of experts from Bulgaria, Poland, Russian Federation and F.R. Yugoslavia. The group members from Croatia and

Romania were also invited, but apologized for being unable to attend. Corresponding members from the Czech Republic and Slovakia were thanked for sending information on their national collections.

The coordinator briefly explained the history, objectives and mode of operation of ECP/GR and mentioned the Vegetables Network's activities carried out and planned within Phase VI of ECP/GR (1999-2003). In particular, he summarized the recommendations made by the Vegetables Network Coordinating Group (NCG) during its meeting in Vila Real, Portugal, in May 2000.[1] *Inter alia*, the Working Group was invited to: acquire information from more countries on the status of *Brassica* genetic resources in Europe; complete the database with missing data sets; finalize the minimum descriptors revised list; include minimum characterization data in the database; compile, agree and implement regeneration guidelines; and ensure systematic and organized safety-duplication of the collections.

He then indicated that the next opportunity for the *Brassica* WG members to meet would be during the Vegetables Network meeting planned for 2003 with the objective of reviewing progress made by the Network and planning for its future.

L. Maggioni gave a brief account of the outcomes of the mid-term meeting of the ECP/GR Steering Committee held in St. Petersburg, Russian Federation, on 14-17 October 2001. Several policy issues were addressed; however no specific Material Transfer Agreement model was endorsed. It was preferred to wait for the adoption of the revised International Undertaking (IU), which was thought to be imminent.[2] A statement was made on the IU negotiations, recommending the extension of the list of crops to be included in a Multilateral System. Regarding the Vegetables Network, the Committee endorsed the establishment of a new Working Group on Solanaceae and invited the group to meet jointly with the next meetings of the GEN RES project EGGNET. It was also suggested to organize an *ad hoc* meeting on Cucurbits[3] and the Vegetables NCG was invited to prepare a proposal for Working Groups on Leafy Vegetables and on Cucurbits, to be considered for approval by the Steering Committee in October 2003.

The Steering Committee was also pleased to see progress made for all ECP/GR objectives, but recommended increased attention to facilitate utilization of plant genetic resources (PGR) in Europe and to increase awareness on the importance of PGR conservation and use. In order to develop a strategy for the next Phase VII, two task forces composed of a few Steering Committee members were established to discuss (1) the impact on PGR of recent developments in science, technology and international policy; and (2) how genebanks should implement relevant international agreements and their impact on their operation.

EPGRIS—European Plant Genetic Resources Information Infra-Structure

Eliseu Bettencourt, national coordinator from Portugal and member in the EU-funded EPGRIS project, explained that this 3-year project (2000-2003), developed within the ECP/GR Documentation and Information Network, was approved for funding within the Fifth Framework Programme of the European Union. The objective is to establish a European

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1 Maggioni, L. and O. Spellman, compilers. 2001. Report of a Network Coordinating Group on Vegetables. *Ad hoc* meeting, 26-27 May 2000, Vila Real, Portugal. International Plant Genetic Resources Institute, Rome, Italy.

2 On 3 November 2001, the renegotiation of the FAO International Undertaking came to an end. The revised text, adopted through a vote, is called "International Treaty on Plant Genetic Resources for Food and Agriculture" (http://www.fao.org/AG/cgrfa/itpgr.htm). This new legally binding international agreement will enter into force when ratified by at least 40 states. The Treaty establishes a Multilateral System ensuring facilitated access to plant genetic resources for food and agriculture. The system covers a specific list of crops, also including the Brassica complex. Genera included are *Brassica*, *Armoracia*, *Barbarea*, *Camelina*, *Crambe*, *Diplotaxis*, *Eruca*, *Isatis*, *Lepidium*, *Raphanobrassica*, *Raphanus*, *Rorippa* and *Sinapis*. The species *Lepidium meyenii* (maca) is excluded.

3 An *ad hoc* meeting on Cucurbit genetic resources was held in Adana, Turkey, 17-19 January 2002.

Internet Search Catalogue (EURISCO) with passport information for plant genetic resources maintained *ex situ* in Europe. The catalogue will be frequently updated and publicly accessible via the Internet. Initial data sets will be derived from the European Central Crop Databases (ECCDBs); however the project will promote the creation of national inventories, which are planned to become the main source of data. PGR National Coordinators of the majority of European countries have nominated national inventory focal persons. These people will be invited to attend three subregional meetings to discuss coordination and standardization of the data flow from the national inventories to the central catalogue. The project partners will also provide technical support to the focal persons and a limited number of training visits to the main European documentation support centres will be arranged. EURISCO will carry an important minimum set of passport data, frequently and automatically updated from the national inventories. A Web-based interface will allow easy searching of the European national inventories, in the same way as it is possible today to use SINGER (System-wide Information Network for Genetic Resources) to search the CGIAR collections and GRIN (Genetic Resources Information Network) to search the USDA collections.

EURISCO can be seen as an important European contribution to the Clearing House Mechanism (CHM) of the Convention on Biodiversity and the implementation of the Global Plan of Action.

E. Bettencourt went on to explain that the project would use the revised version of the FAO/IPGRI Multi-crop passport descriptor list (MCPD), recently finalized (December 2001).

He illustrated the new descriptors, such as collecting institute code, species authority, subtaxa authority, common crop name, acquisition date, breeding institute code, some changes in the biological status of the sample (former "sample status"), ancestral data and some changes in the collecting/acquisition source. The full list is available on-line (http://www.ipgri.cgiar.org/publications/pubfile.asp?id_pub=124).

He also explained the implications for the central *Brassica* Database (Bras-EDB). The idea will be to take the work of collecting the passport data away from the central crop database manager, since these data will become directly accessible from the EURISCO catalogue. On the other hand, ECCDB managers will be expected to dedicate more time to compile and analyze characterization and evaluation data.

Discussion

Loek van Soest asked whether the existing Bras-EDB data would be transferred to EURISCO. Dave Astley seconded this question and asked about the conversion of the existing data into the new MCPD format.

E. Bettencourt replied that the first set of data used by EURISCO would indeed be taken from the central crop databases. Once EURISCO became fully operational, data would be automatically uploaded from the national inventories. He also clarified that the EPGRIS project would take care of the conversion into the new MCPDs. Moreover, the maintenance of the EURISCO catalogue will be guaranteed by IPGRI after the end of the project, while maintenance of the national information systems will remain the responsibility of the individual countries.

G. Thomas expressed some worries about the possible duplication of effort dedicated to developing the national system and the existing European Bras-EDB.

On the technical aspects, Gert Poulsen confirmed that a crop-type descriptor was needed.

Mats Gustafsson pointed out that the passport data were of minor interest for breeders and that the characterization data were the most useful. These would only be stored in the Bras-EDB.

L. van Soest suggested that a central system would risk generating many seed requests sent directly to IPGRI rather than to the collection holders. He wondered whether IPGRI was ready to deal with these practical requests. L. Maggioni answered that this could be

taken into account by IPGRI. He also emphasized the main advantage of the project for genebank curators, who would only have to send a single multi-crop updated file to their national inventory.

Review of data in the Central *Brassica* Database and provision for further data transfer

New version of the European Brassica *Database*

Loek van Soest, on behalf of Ietje Boukema, explained that the European Bras-EDB was established in 1993 in the framework of the ECP/GR Working Group on *Brassica*. By September 1997 the Bras-EDB included 13 000 accessions from 21 collections of 17 European countries. Updating the 1997 version of the Bras-EDB was possible with funding from the EU GEN RES CT99 109-112 project on *Brassica*.

Institutions in Europe holding *Brassica* collections were requested to send new data sets to CGN. New data sets were received from 29 institutions. All the data sets were imported in MS Access and this database was made searchable on-line and downloadable via the Internet.

The new version of the Bras-EDB includes data sets from 32 different institutions in 22 countries (mainly European), with a total of 19 113 accessions. This means an increase of 6113 accessions compared with the 13 000 accessions of the 1997 version of the Bras-EDB. The new accessions have the following origin:

- Contribution of collections from countries previously not represented (e.g. Austria, Romania, Slovakia, Ukraine and Yugoslavia)
- Contribution of new collection holders from countries previously already included (e.g. Germany, Portugal and Spain)
- Increase of collections already presented in the version established in 1997.

Germany holds the largest number of accessions (4212), followed by UK (3108), Spain (1917), the Netherlands (1377), Czech Republic (1211) and Russia (1040). *Brassica oleracea*, a species with several economical important crop types, is, with over 10 000 accessions, the largest species collection. Other relatively large species collections are *B. napus* (rapeseed) and *B. rapa* (turnip and turnip rapeseed) with 3787 and 3022 accessions respectively. A number of wild *Brassica* species are only represented in the Bras-EDB with a few accessions. *B. cretica* is an exception with 112 accessions.

Tracing duplicates is now possible, but is beyond the present capacity of CGN. However, collection holders can check with the Bras-EDB whether accessions in their collection are unique and prioritize these for necessary regenerations.

All data donors are requested to check whether the transformation of their data was done correctly.

The new version of the Bras-EDB can be found on CGN's homepage (http://www.cgn.wageningen-ur.nl/pgr/).

Noor Bas then gave an on-line demonstration showing how the European *Brassica* Database can be searched to retrieve accession data. She encouraged users to send their feedback to the database manager, who would welcome any suggestion to improve the user-friendliness of the database.

GIS analysis of the Brassica *database*

L. Maggioni presented the DIVA-GIS software, a tool developed at the International Potato Center (CIP) in collaboration with IPGRI and with additional financial support from the

System-wide Genetic Resources Programme (SGRP) and others.[4] The software can assist in spatial analysis to identify areas of high diversity, to identify gaps in a collection, to target genetic resources for breeding programmes and to select sites for *in situ* conservation. For demonstration, data downloaded from the on-line European *Brassica* database (Bras-EDB) were plotted on a map with DIVA. Latitude and longitude data were available for 2349 accessions out of a total of 19 113 accessions (= 12%). The most significant fraction of these data was for 1807 landraces (43% of the total landrace data stored in the Bras-EDB) and these were used for subsequent analysis. Maps showing the distribution of the various *Brassica* species and subspecies were presented. Among the features of the software which were demonstrated was the possibility of checking the accuracy of the coordinates attributed to each accession, whether they correctly fall inside the polygons of the continents or inside the boundaries of the respective countries of origin. Points on the map that do not match the above relations are easily identified. A list of apparently mismatched accessions was made available to the Group. Different maps can be overlaid and offer the possibility of finding significant correlations between the spatial distribution of the accessions and altitudes, climatic data, etc. The analysis of the available data for diversity richness identified a hot spot of *Brassica* species richness in western Ukraine and areas with richness of *Brassica oleracea* subspecies in eastern Hungary and northern Portugal. However, these findings could only show the analytical potential of the software, but had no absolute value, due to the limited coverage of spatial data presently recorded in the Bras-EDB. DIVA also allows for the determination of various diversity indices and the distribution of useful traits. Obviously, a full coverage and accuracy of passport and characterization data, including molecular data, would be needed to maximize the analytical potential of GIS software.

Discussion

Tatjana Sretenović-Rajičić, F.R. Yugoslavia and Iwona Bartkowiac Broda, Poland, said that additional data for their respective national collections would soon be sent to the *Brassica* DB manager for inclusion in the Bras-EDB.

In answer to a question from G. Poulsen, G. Thomas explained that there were no data from France on *B. napus*, since the collection is very limited. He informed that networks for each species, with members from both private and public institutions and involved in PGR, were being established in France. These would permit the inclusion of new data for *B. oleracea* (200) and *B. napus* before 2003.

Stefan Neykov explained that, apart from the *Brassica* collection stored in Sadovo, Bulgaria, other collections, mainly of breeding material, were present in other vegetable research institutes in Bulgaria. He said that part of this material would be incorporated in the Sadovo collection and the related data would be sent to the Bras-EDB.

M. Gustafsson wished to thank Bulgaria for their continuing contribution to the development of the Bras-EDB, which includes around 900 accession data from this country. On the other hand, he expressed concern about the small amount of data received from Austria and encouraged the Group to ask for a more complete data set from this country.

It was later specified by I. Boukema that Arche Noah (Austria) would provide the passport data for their *Brassica* collection as soon as the conversion into the appropriate database format is completed. However, this process is progressing slowly due to labour shortage.

The Group considered that the first set of characterization data to be included in the Bras-EDB would be the minimum set in the format to be agreed within the GEN RES project. This format could be used as a test and subsequently offered to the other members of the WG for comment and possible adoption for inclusion of additional characterization data from other Group members.

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[4] DIVA-GIS is available free of charge from http://gis.cip.cgiar.org/gis/tools/diva.htm

Workplan

- *All Working Group members are invited to download from the Bras-EDB their respective country data, check for data accuracy and data completeness and send any correction needed to the DB manager* ***before 31 December 2002****.*
- *All Working Group members are invited to send missing passport data sets to the Bras-EDB. A specific invitation is addressed to the member from Austria that a more complete documentation of the country's collection be sent to the Bras-EDB.*
- *Before the end of 2003, the Bras-EDB database manager will:*
  - *Update addresses and address codes of the data donors*
  - *Publish on the Web site a table with the mailing addresses of the data donors, in order to avoid requests for material from other collections being sent to CGN*
  - *Update the field for common names*
  - *Improve on-line searching by making it more user-friendly*
  - *Mark accessions belonging to the four core collections (*B. oleracea, B. rapa, B. napus *and* B. carinata*) established in the EU GEN RES project.*
- *L. van Soest will, as one of his tasks for the GEN RES project, prepare a format for characterization data exchange, to be tested by the EU project partners. At the latest* ***before the end of 2003****, the agreed format will be circulated to all Working Group members for comments and possible use.*
- *All members were invited to give the state of characterization and evaluation data in each collection. Non-attending members will also receive a questionnaire to be returned to G. Thomas* ***no later than 30 October 2002.***

Review of methodologies for safety-duplication and regeneration

Regeneration activities in Bulgaria

Stefan Neykov explained that regeneration activities were very limited due to lack of resources. The normal practice includes the use of bees as well as hand-pollination of *B. oleracea*.

Regeneration activities in Italy

Ferdinando Branca explained that in Italy *Brassica* germplasm is mainly conserved by the national genebank located in Bari at the Istituto del Germoplasma (IDG) which belongs to the National Research Council (CNR), and by six other institutions - three in Sicily and the others in the Marche, Toscana and Umbria regions. Most of the *Brassica* germplasm is conserved by "Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari" (DOFATA), University of Catania (active collections of 457 accessions of cultivated and wild species) and by the Botanical Garden of Palermo University (wild species and related herbarium).

In order to maintain the original genepool, regeneration of *Brassica* species, which are often self-incompatible, requires specific management for controlled pollination, either obtained by spatial isolation in the field or by the use of isolation chambers.

Of about 1500 accessions conserved in Italy, about 10% have been regenerated so far. In the last few years, DOFATA has increased the regeneration activity in order to undertake characterization and evaluation work. The protocol in use involves sowing in trays in greenhouses and transplanting plantlets at the 3-4 leaf stage into 10-litre pots filled with peat and sand (1:1 in volume). Plants (30-50 per accession) are then grown in the open until the flowering stage and then moved to isolation chambers either in cold greenhouses or in the field. Flesh flies (*Sarcophaga carnaria*) are also used, after raising them in peat from larvae to

the adult stage. Particular attention is paid to synchronize metamorphosis with plant flowering. The flies reach the adult stage in 8 and 18 days at 28ºC and 13ºC respectively.

At the end of the flowering stage, plants are moved to the field to complete the ripening stage utilizing all available solar radiation.

Seed amounts harvested per plant average 28.4 g for cauliflower, 21.8 g for kale and 8.8 g for some wild *Brassica* species.

Regeneration and safety-duplication activities in Poland

Iwona Bartkowiak Broda reported that regeneration in Poland is carried out by a breeding company and that half of the *Brassica* collection is regenerated yearly. Single plants are isolated and individually harvested. A selection of regenerated *B. napus* accessions are sent to Wageningen, the Netherlands, as safety-duplicates.

Safety-duplication and regeneration activities in the Russian Federation

Anna Artemyeva explained that all *Brassica* collections are stored in St. Petersburg in aluminium boxes at room temperature. Between 1965 and 1995, 61% of the accessions in the base collection of *Brassica* were duplicated in St. Petersburg at 4°C under vacuum in glass jars (3000 seeds). Since 1996, 34% of the accessions in the *Brassica* base collection were duplicated in VIR in refrigerators at –10°C for long-term storage.

New cold storage equipment, recently built in St. Petersburg will host the working (active) collections at 4°C in laminated aluminium packages for short-term storage.

Since 1975, 57% of the accessions in the base collection of *Brassica* crops were duplicated in the National Seed Storage at the Kuban experimental station at 4°C in glass jars (6000 seeds per accession, with about 6% seed moisture). Finally, 8% of the accessions in the base *Brassica* collection were duplicated in the VIR genebank in refrigerators for long-term storage at -10°C in laminated aluminium packages (6000 seeds per accession). The new equipment at St. Petersburg, will host a duplicate of the whole base collection of VIR, at -10°C in laminated aluminium packages for long-term storage.

The accessions of the *Brassica* collection are sown every fourth or fifth year for regeneration at eight experimental stations of the Vavilov Institute, with the exception of the accessions of the "passive" part of the collection duplicated in long-term storage in St. Petersburg.[5] The criterion for regeneration is a germination rate below 50%.

A few accessions are regenerated on isolated plots (60-100 plants, with natural pollination by honey bees and flies). Many accessions are regenerated in isolation chambers and some accessions in polythene tunnels (30-40 plants, with pollination by hand or by bees or flesh flies). Fifty percent of the accessions in the vegetable *Brassica* collection are regenerated during winter in southern Russia.

Safety-duplication and regeneration activities in F. R. Yugoslavia

Tatjana Sretenović-Rajičić stated that a project for the safety-duplication of all the national collections has been planned in F.R. Yugoslavia. For the year 2002, the plan foresees safety-duplication of 15 accessions from the Brassicaceae collection (domestic populations of cabbage). The main concern is to preserve samples from accidents such as the flood in 1999, when some accessions included in the regeneration process in glasshouses or stored in the basement genebank chamber were seriously damaged.

Protocols for the regeneration of most samples have been established and consist of planting in the field and transplanting into greenhouses. Honey bees are used for pollination and chemical treatments are applied. *Raphanus* is regenerated in the field with a separation of 2000 m, using honey bees. On the other hand it is very difficult to regenerate

[5] The "passive" part of the collection includes accessions under study, and the value of which has not yet been established or confirmed.

cauliflower which flowers but does not set seed. The criterion for regeneration is a germination rate below 75%.

Discussion on safety-duplication

L. van Soest informed the Group that safety-duplication is also a task for the GEN RES project. A table specifying the conditions for storage of safety-duplicates was prepared by the project partners and circulated to the Group for them to add further information. It was considered that bilateral arrangements for safety-duplication should be encouraged and established on the basis of available space in genebanks with appropriate conditions for long-term storage.

Recommendation

The Working Group recommends that bilateral arrangements be made in order to maximize the level of safety-duplication of the collections. In particular, every time that material is regenerated, a sample should immediately be sent as a "black box" to a different genebank, possibly in a different country.

Regarding technical aspects:

- *Samples sent for safety-duplication should contain a minimum of 300 seeds*
- *The bilateral arrangement should be made with institutes offering optimum long-term storage conditions (-20°C, etc.).*

Discussion on regeneration

The Group discussed whether it would be appropriate to try to define guidelines for regeneration procedures. M. Gustafsson reminded the Group that research on routine regeneration practices of European genebanks had shown the occurrence of allele shifts, misplacement of samples and introduction of new alleles. Although there is still limited scientific background to establish objective criteria to adopt for the regeneration of each *Brassica* species, the Group felt that at least some minimum guidelines should be formulated, in order to improve the quality of the regeneration methodologies.

It was also noted that theory requires that 200-300 plants be used to regenerate each accession, in order to minimize the loss of rare alleles. According to Crossa (1998)[6] a sample size of 130-200 individuals will give a high probability of retaining rare alleles at low frequency in most of the loci. However, in practice, most genebanks cannot afford to use more than 50-60 plants in isolation cages and it is not feasible to aim at conserving the rare alleles. Les Breese gave an excellent overview in a 1989 IBPGR booklet.[7]

Recommendation

The Group agreed that the following minimum guidelines should be followed for the regeneration of Brassica *accessions:*

1. *Use no less than 50-60 plants per accession*
2. *Undertake either controlled pollination inside isolation cages or tunnels (making use of pollinator insects), or allow for outdoor open pollination, with a minimum separation distance*
3. *Give priority to regeneration of unique accessions of national origin. The Bras-EDB should be checked to verify the uniqueness or the level of duplication of accessions in the European collections.*

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[6] Crossa, J. 1998. Sample size and effective population size in seed regeneration of monoecious species. Pp. 140-143 *in* Regeneration of seed crops and their wild relatives. Proceedings of a consultation meeting, 4-7 December 1995, ICRISAT, Hyderabad, India (J.M.M. Engels and R. Ramanatha Rao, eds). International Plant Genetic Resources Institute, Rome, Italy.

[7] Breese, E.L. 1989. Regeneration and multiplication of germplasm resources in seed genebanks: The scientific background. International Board for Plant Genetic Resources. Rome, Italy.

Workplan

In order to survey the current regeneration procedures used in European genebanks, G. Thomas will prepare a questionnaire and will send it to all the WG members by the end of March 2002. The results will be published in the final report.[8]

G. Thomas circulated a table to be filled by the Working Group members, to indicate the number and % of accessions regenerated each year. Together with the questionnaire, the table will also be circulated to the other members of the Group, so as to have an overview of the state of regenerated collections in the Working Group.

All members are expected to return the above information to G. Thomas no later than ***30 October 2002.***

In situ conservation actions

Lorenzo Maggioni reminded the Group of the activity of a task force on wild species conservation in genetic reserves, which was established during a meeting of the ECP/GR *In situ* and On-farm Conservation Network at Isola Polvese, Italy, in May 2000.[9] Among its recommendations, the task force stressed the need to develop two projects focused on genetic reserve conservation of wild cereals (wheat, barley and oats) and of wild *Brassica* species in Europe. This last proposal followed previous suggestions made by the Working Group on *Brassica* that Sicily would be the priority area for *in situ* conservation, considering its *Brassica* species diversity and their endemic status.[10] A project on "*In situ* genetic conservation of *Brassica* species in European genetic reserves" drafted by Nigel Maxted and Mats Gustafsson was circulated to the Group in advance of the meeting and the Group was asked to comment on its content and to brainstorm on a possible strategy to promote concrete action.

The draft project from Maxted and Gustafsson proposes to collate existing ecogeographic data and analyze them, using GIS techniques. The project aim is to define locations for and establish *in situ* reserves for the *Brassica* genepool in Europe.

The most appropriate funding source for a European collaborative project has yet to be identified. A proposal for a new EC regulation (ex 1467/94) (http://europa.eu.int/eur-lex/en/com/reg/en_register_033050.html) is under discussion in Brussels and is expected to be launched in 2002. The aim of the regulation will be to finance measures to promote the conservation, characterization, collection and utilization of genetic resources in agriculture.

Also the EU Sixth Framework Programme of the European Community for research, technological development and demonstration activities is planned to start in 2002. In the current proposal, the priority thematic area "Global change and ecosystems" includes, *inter alia*, the research objective of preserving ecosystems and protect biodiversity, which would also contribute to the sustainable use of land and marine resources. New instruments are envisaged for the implementation of this programme, in particular through networks of

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[8] This action was not completed due to the low response received before the publication of the present report. The full information is planned to be collected in time for the Vegetables Network meeting to be held in May 2003.

[9] Laliberté, B., L. Maggioni, N. Maxted and V. Negri, compilers. 2000. ECP/GR *In situ* and On-farm Conservation Network. Report of a joint meeting of a Task Force on Wild Species Conservation in Genetic Reserves and a Task Force on On-farm Conservation and Management, 18-20 May 2000, Isola Polvese, Italy. International Plant Genetic Resources Institute, Rome, Italy.

Maggioni, L., D. Astley, M. Gustafsson, T. Gass and E. Lipman, compilers. 1997. Report of a Working Group on *Brassica*, Third meeting, 27-29 November 1996, Rome, Italy. International Plant Genetic Resources Institute, Rome, Italy.

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. International Plant Genetic Resources Institute, Rome, Italy.

excellence aiming at advancing knowledge in a particular area by assembling a critical mass of expertise. Activities will be generally targeted towards long-term, multidisciplinary objectives, rather than predefined results in terms of products, processes or services.

L. Maggioni concluded by suggesting that a possible theme for a research proposal to be carried out by a network of excellence could perhaps be the "Understanding and protection of the genetic diversity of wild relatives of agricultural crops in Europe".

Discussion

Several members illustrated actions regarding *in situ* conservation:

F. Branca mentioned an Italian project proposal on the investigation of the diversity of traits (glucosinolates) and the gene flow between wild species and landraces in Sicily.

N. Stavropoulos emphasized the difficulty in obtaining a consensus from the local or national communities. This requires visiting the local populations and making them proud of their genetic wealth. The interest of the EU will be both to maintain the farming practice and the *ad hoc* landraces.

T. Sretenović-Rajičić indicated that wild species in Montenegro should be investigated and has already identified interest in landraces of Brassicaceae species with traits such as herbicide resistance.

E. Rosa mentioned ongoing activities in Portugal together with Italy, UK and USA to screen wild species for evaluation of glucosinolates in broccoli, with the purpose to breed varieties that can be used as health products or for intercropping in a more sustainable agricultural system.

G. Thomas said that investigations of diversity have been carried out for many species in many research projects, but actual protection in genetic reserves is not advanced. On this point, priorities in France were established on *Beta*, *Brassica* and *Olea*.

Considering the global *in situ Brassica* project written by N. Maxted and M. Gustafsson, G. Thomas asked the Group to identify priorities among the five tasks proposed.

The Group agreed that the protection of genetic reserves (whatever the species) must be under the control of the local authorities (state, region, etc.).

L. van Soest expressed interest in the task of increasing the complementarity between *in situ* and *ex situ* conservation. This requires defining uses for wild *Brassica* germplasm and collecting and regenerating the material.

M. Gustafsson said that seed could be made available either from regeneration of *ex situ* material or from *in situ* natural sources. He thought that the Group needed to seek the opinion of the breeders.

I. Bartkowiak Broda, M. Binnendijk and W. Lühs confirmed that wild species for pest and disease resistance could be very valuable for the improvement of *B. oleracea*, *B. napus* and *B. rapa*.

Recommendations

The Group recommended undertaking an action in two stages:

- *First, to confer with European breeders in order to identify a list of traits showing lack of variability and for which genetic introgression of wild* Brassica *would be interesting*
- *Secondly, for the main traits identified, to plan and establish common evaluation tests of the whole set of wild* Brassica *contained in the European collections (according to Bras-EDB data). Funding would need to be sought for common action between European breeders and the Group (future GEN RES project).*

Strengths and weaknesses in *Brassica* genetic resources work in each country

G. Thomas asked the Group members to indicate the main strengths and weaknesses of their national systems with regard to the conservation of *Brassica*. Replies are compiled in Table 1 below.

Table 1. Strengths and weaknesses in *Brassica* conservation in European countries

	Strength	Weakness
Bulgaria	Recent important inclusion of updated data in Bras-EDB	Continue the collecting at the international level
France	Originality of the collection	Only one official collection (a network including all PGR partners is being established and will produce a nationally shared collection)
Germany	Material with high germinability Efficient regeneration system, with use of own pollinator insects Taxonomy expertise	Regeneration capacities (limited number and size of isolation cages)
Greece	Establishment of a national PGR programme	Funding for staff and facilities Lack of collecting on the Ionian coast and Northern Greece
Italy	Informal network for conservation and exploitation of vegetable *Brassica* established	Updating of passport data
Netherlands	On-line provision of passport, characterization and evaluation data Good system for regeneration and conservation	Some missing passport data need to be added using other databases and variety lists
Nordic countries	Nordic Gene Bank data information system	
Poland	Originality of the collection (local populations)	Funding for evaluation (field and vegetable *Brassica*)
Portugal	Good storage conditions and available storage capacity	Regeneration possibilities at the genebank in Braga
Russian Federation	Wide diversity mainly originated from Russia and former Soviet Union	Regeneration capacity
Spain	Originality of the collections: wild brassicas, local populations	Limited facilities and funds for regeneration
United Kingdom	Good integrated management system for conservation and regeneration within the constraints of technical facilities and staffing. The new Genomic Centre for Sustainable Horticulture will provide additional resources	*Brassica* is in competition for resources/facilities for regeneration and characterization with other outbreeding crops, such as *Alllium* and *Daucus*
F.R. Yugoslavia	Amount of diversity (partially unexplored)	Limited regeneration capacity, storage space and storage conditions (+4°C)

G. Thomas asked T. Sretenović-Rajičić whether she would agree to act as the focal person in her area for contacts between the Group and the members of the neighbouring countries, in case of need.

Conclusion

Presentation and adoption of the report

The section *Discussion and Recommendations* of the report was presented to the participants and was approved with minor modifications.

Closing remarks

The Group wished to thank Eduardo Rosa for the excellent organization of the meeting and for sharing his knowledge of local traditions, foods and beverages.

PART II. PRESENTED PAPERS - ECP/GR SESSION

The European *Brassica* Database: version 2001 **14**
Characterization, evaluation, regeneration and other activities of the Bulgarian Cruciferae collection **19**
Quality of Bulgarian cabbage cultivars **22**
Status of the Czech national *Brassica* collection **24**
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Status report update on the *Brassica* collections in German genebanks **27**
***Brassica* regeneration activities carried out in Italy** **29**
Status of *Brassica* germplasm collections in Poland **32**
Status of the *Brassica* collection in Russia **34**
The *Brassica carinata* (Braun) collection in Russia **38**
Identification of *Brassica* species by storage seed proteins and evolution of the genus *Brassica* **39**
Old local Russian cultivars of white cabbage **43**
Report on the Brassicaceae collection in Yugoslavia **45**

The European *Brassica* Database: version 2001

Ietje W. Boukema[1], Neculai Cristea[2], Theo J.L. van Hintum[1] and Frank Menting[1]

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[2] *Suceava Genebank, Suceava, Romania*

Introduction

The European Bras-EDB was established in 1993 by the Centre for Genetic Resources, the Netherlands after a decision of the ECP/ GR *Brassica* Working Group meeting in 1991 in the Czech Republic (Hintum and Boukema 1993). The objectives of this database are to support rationalization of *Brassica* germplasm conservation and improve the access of this germplasm by users. The database includes cultivated as well as wild material. By September 1997 the Bras-EDB included 13 000 accessions from 21 collections of 17 European countries (Boukema and Hintum 1998). The updating of the database in 2001 increased this number to 19 113 accessions

Methods

Institutions in Europe holding *Brassica* collections were requested to send new data sets to CGN. New data sets were received from 29 institutions. The data of the old Bras-EDB were maintained for 3 collections because no new data were received. The data were imported per data set in MS Excel and transformed to fit the FAO/IPGRI multi-crop passport descriptors. Some additional passport descriptors were used. Much effort was given to transforming taxonomic names, as received from the collection holders, into a uniform system. Finally all the data sets were imported in MS Access and this database was made searchable on-line and downloadable via the Internet (www.genebank.nl/brasedb).

Results

The new version of the Bras-EDB presently includes data sets from 32 different institutions of 22 mainly European countries (Table 1), with a total number of 19 113 accessions. This is an increase of 6113 accessions compared with the 13 000 in the 1997 version of the Bras-EDB. The new accessions have the following origin:

- contribution of collections from countries previously not represented (e.g. Austria, Romania, Slovakia, Ukraine and Yugoslavia F.R.)
- contribution of new collection holders from countries previously already included (e.g. Germany, Portugal and Spain)
- increase of the number of accessions in collections already present in the 1997 version.

Table 1 shows that Germany holds the largest number of accessions (4212), followed by UK (3108), Spain (1917), the Netherlands (1377), Czech Republic (1211) and Russia (1040). An overview of the number of accessions per species is presented in Table 2, which shows that *B. oleracea,* a species with several economically important crop types, is the most represented species with over 10 000 accessions. Other relatively important species are *B. napus* (rapeseed) and *B. rapa* (turnip and turnip rapeseed), commonly cutivated in Europe, with 3787 and 3022 accessions respectively. Table 2 also indicates that a number of wild *Brassica* species are only represented in the Bras-EDB by a few accessions. *B. cretica* forms an exception with 112 accessions. Table 3 gives the number of accessions per major taxonomic group and data source.

Table 1. Number of accessions per country and institution

Country	Total per country	Institution	No. of accessions
Austria	14	Federal Office and Research Centre for Agriculture, Vienna	2
		Federal Office of Agrobiology Seed Collection, Linz	10
		National Institute of Plant Breeding and Seed Testing, Rinn, Tirol	1
		Research Station for Special Crops, Wies	1
Belgium	163	Government Plant Breeding Station (RVP), Merelbeke	163
Bulgaria	895	Institute of Plant Genetic Resources (IPGR),Sadovo, Plovdiv	895
Czech Republic	1211	Research Institute for Crop Production (RICP), Prague-Ruzyne	1211
France	847	Station d'amélioration des plantes, INRA, Le Rheu, Rennes	847
Germany	4212	Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben	1975
		Institute for Plant Genetics and Crop Plant Research (IPK), Malchow	1048
		Federal Centre for Breeding Research on Cultivated Plants (BAZ), Braunschweig	1189
Greece	169	Greek Genebank (GGB), Agricultural Research Centre of Makedonia, Thessaloniki	169
Hungary	160	Institute for Agrobotany (RCA), Tápiószele	160
Italy	528	Istituto del Germoplasma (IDG), Bari	528
Netherlands	1377	Centre for Genetic Resources, the Netherlands (CGN), Wageningen	1377
Nordic countries	477	Nordic Gene Bank (NGB), Alnarp, Sweden	477
Poland	728	Plant Breeding and Acclimatization Institute (IHAR), Blonie, Radzikow	728
Portugal	898	Instituto Superior de Agronomia (ISA), Lisboa, Portugal	51
		Banco Portugues de Germoplasma Vegetal (BPGV), Braga	847
Romania	109	Genebank of Suceava	109
Russia	1040	N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg	1040
Slovakia*	112	Research Institute of Plant Production (RIPP), Piesťany	112
Spain	1917	Mision Biologica de Galicia (MBG), Pontevedra	375
		Banco de Germoplasma de Horticolas (BGH), Zaragoza	577
		Centro de Recursos Fitogeneticos (CRF), Alcala de Henares, Madrid	339
		Department of Plant Biology, School of Agriculture, Polytechnical University of Madrid (UPM)	239
		Genebank of the Polytechnical University of Valencia (UPV)	387
Switzerland	122	Station Fédérale de Recherches Agronomiques de Changins (RAC), Nyon	122
Turkey	302	Plant Genetic Research Department, Aegean Agricultural Research Institute (AARI), Izmir	302
Ukraine	542	Yurjev Institute of Plant Breeding (NCPGRU), Kharkov	542
United Kingdom	3108	Genetic Resources Unit, Horticulture Research International (GRUHRI), Wellesbourne	3108
Yugoslavia	182	Vegetable Research Centre, Smederevska Palanka (CFVCSP)	182
Total	**19113**		**19113**

* data from the Research Institute of Vegetables not yet included

Tracing duplicates

Tracing duplicates is now feasible, but is beyond the present capacity of CGN. Collection holders can find out from the Bras-EDB whether accessions in their collection are unique and prioritize these for regeneration.

Future activities

Some minor improvements are still needed:

- Inclusion of additional data sets (e.g. from the Research Institute of Vegetables, Slovak Republic and from Arche Noah, Austria)
- Updating of the field for common names
- Updating of addresses and address codes of the data donors
- Inclusion of a table with the mailing addresses of the data donors to avoid seed requests to CGN for material from other collections
- Marking accessions belonging to the four core collections (*B. oleracea*, *B. rapa*, *B. napus* and *B. carinata)* established in the EU GEN RES project.

Table 2. Number of accessions per species

Genus	Species	No. of accessions
Brassica	*balearica*	3
Brassica	*barrelieri*	5
Brassica	*bivoniana*	1
Brassica	*bourgeaui*	4
Brassica	*carinata*	310
Brassica	*cretica*	112
Brassica	*deflexa*	2
Brassica	*desnottesii*	1
Brassica	*drepanensis*	4
Brassica	*elongata*	9
Brassica	*fruticulosa*	19
Brassica	*gravinae*	7
Brassica	*hilarionis*	4
Brassica	*incana*	39
Brassica	*insularis*	26
Brassica	*juncea*	647
Brassica	*macrocarpa*	12
Brassica	*maurorum*	5
Brassica	*montana*	46
Brassica	*napus*	3787
Brassica	*nigra*	298
Brassica	*nivalis*	1
Brassica	*oleracea*	10252
Brassica	*oxyrrhina*	1
Brassica	*procumbens*	1
Brassica	*rapa*	3022
Brassica	*repanda*	12
Brassica	*rupestris*	28
Brassica	*souliei*	4
Brassica	*spinescens*	1
Brassica	*tournefortii*	17
Brassica	*villosa*	25
Brassica	unknown	397
x *brassicoraphanus*		11
Total		**19113**

Acknowledgements

We thank Herman Nijland for his valuable support in building the Internet application. Furthermore we thank all data donors for sending their data and answering our questions. Updating the 1997 version of the Bras-EDB was possible with funding of the EU GEN RES CT99 109-112 project on *Brassica*. The technical work was possible with funds for a visiting scientist from the International Agricultural Centre, Wageningen.

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Table 3. Number of accessions in the Bras-EDB per data source and major taxonomic group

Country	Data source	*B. carinata*	*B. juncea*	*B. napus*	*B. nigra*	*B. oleracea**	*B. rapa*	Wild sp.*	Others and unknown***	Total
Austria	Vienna	-	1	-	1	-	-	-	-	2
	Linz	-	-	1	-	8	1	-	-	10
	Rinn	-	-	-	-	-	1	-	-	1
	Wies	-	-	-	-	1	-	-	-	1
Belgium	RVP	-	-	48	-	22	93	-	-	163
Bulgaria	IPGR	-	18	301	7	489	77	-	3	895
Czech Republic	RICP	27	97	718	28	240	101	-	-	1211
France	INRA, Rennes	-	-	77	-	735	32	-	3	847
Germany	IPK, Gatersleben	37	103	103	17	931	471	97	216	1975
	IPK, Malchow	-	64	643	63	71	191	-	16	1048
	BGRC	115	94	319	77	407	176	-	1	1189
Greece	GGB	-	-	-	-	125	-	43	1	169
Hungary	RCA	1	12	-	-	132	11	4	-	160
Italy	IDG	-	6	13	2	261	166	9	71	528
Netherlands	CGN	108	32	222	24	612	355	5	19	1377
Nordic countries	NGB, Sweden	-	-	191	-	203	83	-	-	477
Poland	IHAR	-	10	219	1	423	57	-	18	728
Portugal	ISA	-	-	-	-	50	1	-	-	51
	BPGV	-	-	34	-	546	259	-	8	847
Romania	Suceava	-	1	79	6	16	2	3	2	109
Russia	VIR	14	-	-	-	980	46	-	-	1040
Slovakia	RIPP	-	-	96	-	16	-	-	-	112
Spain	MBG, Pontevedra	-	-	37	-	187	147	-	4	375
	BGH, Zaragoza	-	-	1	2	456	114	-	4	577
	CRF, Madrid	1	2	27	1	213	51	40	4	339
	UPM, Madrid	-	-	-	-	60	-	179	-	239
	UPV, Valencia	-	-	45	-	321	1	-	20	387
Switzerland	RAC, Nyon	-	-	5	-	99	18	-	-	122
Turkey	AARI	-	-	15	65	194	21	6	1	302
Ukraine	NCPGRU	-	154	231	2	122	33	-	-	542
United Kingdom	GRUHRI	7	53	362	2	2152	512	3	17	3108
Yugoslavia	CFVCSP	-	-	-	-	180	2	-	-	182
Total		**310**	**647**	**3787**	**298**	**10252**	**3022**	**389**	**408**	**19113**

* Including wild *B. oleracea* accessions

** Number of different wild species = 26

*** Including x *Brassicoraphanu;* N.B. For full names of institutes see Table 1.

Characterization, evaluation, regeneration and other activities of the Bulgarian Cruciferae collection

Stefan Neykov, Jordan Angelov and Ivan Lozanov
Institute of Plant Genetic Resources (IPGR), Sadovo, Plovdiv, Bulgaria

During the period 1978-2001, a total of 1196 accessions including 1018 *Brassica* and 178 other cruciferous crops (*Raphanus, Sinapis, Camelina, Crambe* and *Eruca*) were introduced, collected and selected in Bulgaria (Table 1).

The Institute of Plant Genetic Resources (IPGR) in Sadovo maintains 371 accessions of *Brassica oleracea*, including 283 of var. *capitata*. The collection of *Brassica* oil crops consists of 293 samples of *B. napus* subsp. *oleifera,* 105 *B. rapa* subsp. *oleifera,* 45 *B. juncea* and 24 *B. nigra*.

Most of the *Brassica oleracea* accessions were received from HRI (Wellesbourne, UK), CGN (Wageningen, the Netherlands) and VIR (St. Petersburg, Russian Federation).

Many accessions of *B. napus* and *B. rapa* subsp. *oleifera* were introduced from the Czech Republic and Germany. Most of the *Brassica* collection was evaluated for 36 characters according to international descriptor lists (Neykov 1983, 1989; Angelov 1990; Mihov *et al.* 1999, 2000).

Collecting missions in the country resulted in the addition of wild and local forms of *B. rapa, B. nigra, B. juncea, Sinapis alba, Sinapis arvensis, Camelina sativa* (Table 1), *Crambe maritima* and *Conringis orientalis*. Wild forms of *Crambe tataria, Raphanum raphanistrum, R. landra, Brassica jordanoffii, Sinapis nigra*, etc. are also present.

About 50% of the *Brassica* samples are included with characterization data in the Bras-EDB.

Other Bulgarian research institutes also maintain some small *Brassica* collections containing a total of 165 accessions and 105 breeding lines (Table 1).

The collected materials are stored in the Gene Bank in Sadovo, where 691 accessions are kept in long-term storage at -18°C and the others in short-term storage at -4°C.

About 450 seed samples have been distributed for breeding purposes to other institutes and for international exchange.

The *Brassica* accessions are not sent for safety-duplication, as most of them are maintained in CGN and HRI. The *Brassica* local and wild material represents a small part of the collections and will be safety-duplicated.

Old cultivars (especially white and red cabbages) and landraces of *Brassica* can still be found in Bulgaria. Sources of this germplasm are home gardens, mainly at the frontier with F.R. Yugoslavia, as well as in the Rhodope and Belasitsa Mountains, the Black Sea coast, and in northeastern and central-southeastern Bulgaria.

After 1990, agricultural research funding was severely reduced, leading to a decrease in some activities related to collecting, study and regeneration of *Brassica* genetic resources. Even decisions of the Bulgarian Ministry of Agriculture for priority subsidizing of activities related to the collecting and conservation of germplasm were insufficient to improve the work in this field. Recently, for the pollination of cruciferous crops, queen cells were used with bees located on the outside of isolation cages, micro-queen cells with bees, and pollination by flies.

The main *Brassica* genetic resources activities include:

- collecting local landraces and wild relatives and their regeneration
- systematic documentation of the collections and their field evaluation
- long-term storage and *in situ* conservation of genetic resources
- introduction and development of new varieties

- registration of promising cultivars and breeding material with valuable economic characters by the State Cultivar Commission and their utilization.

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

Species	Subspecies/ variety	Collected	Breeders' material	Evaluated/ Characterized	Stored	Regenerated
IPGR-Sadovo						
Brassica oleracea	*capitata*	283	25	283	157	240
	botrytis	47	-	47	-	-
	gemmifera	15	5	14	-	8
	sabauda	9	-	9	5	6
B. rapa	*pekinensis*	14	5	14	5	10
B. campestris	*rapifera*	18	-	16	-	5
B. napus	*oleifera*	293	-	270	270	270
B. rapa	*oleifera*	105	-	45	65	95
B. juncea		45	-	45	30	40
B. nigra		24	-	20	24	24
Camelina sativa		60	-	55	60	60
Crambe abyssinica		20	-	20	18	20
Eruca sativa		3	-	3	3	3
Raphanus sativus	*radicula*	40	-	40	4	15
Sinapis alba		55	-	45	30	55
IVC-Maritsa						
B. oleracea	*capitata*	40	25	40	-	40
	botrytis and others	20	-	-	-	-
BSVC-G. Orjahovitsa						
B. oleracea	*capitata*	60	80	60	-	110
	botrytis and others	15	-	15	-	-
Agricultural University-Plovdiv						
B. oleracea	*capitata*	30	-	30	-	30
Total		**1196**	**140**	**1071**	**671**	**1031**

IPGR = Institute of Plant Genetic Resources
IVC = Institute for Vegetable Crops
BSVC = Breeding Station for Vegetable Crops

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Quality of Bulgarian cabbage cultivars

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Among the *Brassica* crops grown in Bulgaria the most common is the headed cabbage, occupying an area of about 6000 ha and producing 200 000-220 000 tons per year. It is grown for early, medium-early (summer) and particularly for late (autumn) production. Autumn sowing for early spring harvesting is not very common in the country. In terms of cultivated area and total production, the largest share is occupied by late-maturing headed cabbage cultivars (Minkov and Manueljan 1970; Mihov *et al.* 1975; Neykov 1989; Alipieva *et al.* 1999).

The headed cabbage cultivars, grown for summer and late production, are only of local origin (subsp. *orientalis*) (Lizgunova 1959). Cultivars of subsp. *europea* Lizg are grown for early production (Mihov *et al.* 1975).

Taking into account the great diversity and marked differentiation of local cabbage forms, it could be assumed that a secondary centre of origin for oriental cabbages is found on the Balkan Peninsula and Bulgaria in particular (Daskalov and Popov 1949).

A short morphological description will be given for the following local late and medium-late cabbage cultivars.

'Kjosse 17'
Forms large, compact, flat/round (18.5-24.5 cm high and 22.0-28.5 cm wide) heads with mean weight of 3.5-4.5 kg (Table 1), 6.5-8.0% dry matter content, and 4.0-4.6% sugars. Matures earlier than the other cultivars with a growing period of 125 days. Also grown for medium-early production. Suitable for processing and especially for canning as sauerkraut. Resistant to severe summer drought.

'Likorishko bjalo 7'
Leaf rosette - large, 90-105 cm in diameter; stump - medium long (18-20 cm); leaf - lyriform with a medium long petiole; head - medium-sized (2-4 kg), compact, flat/round, white-cream in colour, with a short core (Table 1). This is an old high-yielding cultivar with a growing period of 130-140 days, resistant to hot and dry weather conditions.

'Dabensko' and 'Dabensko 5'
Heads - about 16 cm high and 19 cm wide, medium weight of 2.5 kg; compact (firm) with fine structure and high drought resistance (Table 1).

Table 1. Morphological characteristics of local headed cabbage cultivars

Cultivar	Head					
	Weight (kg)	Height (cm)	Diameter (cm)	Core length (cm)	Compactness (1-5)	Colour
Kjosse 17	3.5-4.5	22-28	15-18	8-10	3-5	white
Balkan	2.5-5.0	16-25	15-20	8-10	4-5	white
Likorishko 7	2.0-4.0	14-18	16-22	8-10	3-4	white
Dabensko 5	1.5-3.5	15-19	14-20	6-9	3-5	white
Marnopolsko	2.0-3.0	17-20	16-19	7-10	4-5	white
Zavidovsko	3.0-4.0	16-19	20-22	7-9	3-5	white
Pazardjishko 16	2.0-4.0	15-19	14-20	6-9	3-5	purple-red

'Balkan'
Stump and leaf rosette - medium-sized; head - large (16-25 cm high and 15-20 cm wide), round, convex in the front, very compact, with a short core (8-10 cm). This is a high-yielding cultivar (4-6 t/da), field resistant to mildew and drought. Growing period 125-130 days.

'Maritsa 48/5' and 'Sredets'
These cultivars develop a medium-sized (70 cm wide) compact rosette. Head flat/round, convex in the front (above the leaves), medium-large (2-4 kg), compact, white-cream in colour and fine structure. Medium-early cultivars (100-125 days), yielding 3-4 t/da, resistant to hot and dry weather.

'Pazardjishko podobreno 16'
Head medium-sized (3-4 kg), compact, white-red-purple in colour, resistant to summer heat and mildew. Average yield of 3-4 t/ha with very good technological properties. The populations 'Sinjo srabsko', 'Melez', 'Sin kapak', and others are similar to this cultivar.

The old local cultivars 'Marnopolsko', 'Zavidovsko', 'Lomsko bjalo', 'Buzovsko bjalo' (some of them distributed after the 18th century in many other countries in the world), also belong to the group of late-maturing cultivars (120-150 days) with very good economic characters. They develop compact heads with fine structure, 2-4 kg weight, with good storability and high heat and drought resistance.

Furthermore, qualities of the Bulgarian cabbage cultivars and populations are also confirmed by the fact that none of the tested foreign cultivars was established and adapted for medium-early and late production in our country. This necessitates the collecting of other local populations and cultivars, their maintenance and conservation.

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Status of the Czech national *Brassica* collection

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The national collection of cruciferous crops in the Czech Republic consists of 1319 accessions including 338 vegetables, 971 oil crops and 10 fodder crops. Vegetable crops are regenerated and evaluated at the workstation of the Research Institute of Crop Production (RICP) Prague-Ruzyne, Gene Bank in Olomouc. The collection of *Brassica* vegetables has been extended since the last revision in 1996 by 152 accessions, mainly advanced cultivars and landraces of cabbage obtained from collecting missions to Slovakia.

Seed production for conservation purposes is carried out in accordance with the CGN's methodology (modified) of using stable isolation cages to avoid contamination by foreign pollen and bees as pollinators. The accessions are progressively evaluated for morphological and agronomic traits and the characterization data are prepared for computerization according to minimal descriptors for inclusion in the Bras-EDB.

The collection of oil crops maintained in the Research Institute of Oilseed Crops (RIOC) in Opava has also been enlarged by about 300 accessions, mostly winter oilseed rape. The regeneration of accessions in the field trials is carried out by means of portable isolation cages.

Passport data for vegetable and oilseed crops are stored in the Czech database EVIGEZ and they are subsequently integrated into the Bras-EDB.

No changes have occurred in the small collection of fodder *Brassica* crops including 10 accessions of fodder kales.

Both Czech main *Brassica* collections in the RICP Genebank, Olomouc station, and RIOC-Opava are gradually regenerated for long-term seed storage in the genebank in RICP, Prague-Ruzyne. For long-term storage the seed samples are dried to a moisture content of about 4%, packed in glass jars and stored at -18°C.

An agreement has been reached with the genebank in the Research Institute of Plant Production (RIPP) in Piešťany (Slovakia) for the safety-duplication of the Czech collection.

The current status of the *Brassica* collections in the Czech Republic is shown in Table 1.

Table 1. *Brassica* accessions in the Czech collection of vegetables, oilseed and fodder crops

Species	Subspecies./ variety	Crop	No. of acc. in collection	Accession status				No. acc. in GB	% of stored acc.
				U	LR	AC.	BR		
RICP Olomouc									
B. oleracea L.	*botrytis*	cauliflower	60			59	1	35	58.3
	capitata	cabbage	169	3	42	121	3	84	49.7
	gemmifera	Brussels sprouts	9			9		6	66.7
	gongylodes	kohlrabi	36			36		20	55.6
	italica	broccoli	3			3		3	100.0
	sabauda	savoy cabbage	15	1		13	1	9	60.0
	sabellica	curly kale	9			9		5	55.6
B. rapa L.	*chinensis*	pak choy	6	6				6	100.0
	rapa	turnip	1				1	1	100.0
B. pekinensis L.		chinese kale	16		10	6		9	56.3
B. napus L.	*napobrassica*	rutabaga	14		1	13		10	71.3
RIOC Opava									
B. napus L.	*napus*	winter oilseed rape	546		1	404	141	525	96.2
		spring oilseed rape	174		1	162	11	173	99.4
B. rapa L.	*oleifera*	winter turnip (oilseed) rape	41	2	2	36	1	41	100.0
		spring turnip (oilseed) rape	41	1		37	3	33	80.5
B. carinata L.		Ethiopian mustard	43	27	16			34	79.1
B. juncea L.		brown mustard	97	38	5	43	11	90	92.8
B. nigra L.		black mustard	29	14		11	4	28	96.6
RIFC Troubsko									
B. oleracea L.	*acephala*	fodder kale	10			10		10	100.0
Total			**1319**	**92**	**78**	**972**	**177**	**252**	**85.1**

RICP = Research Institute of Crop Production
RIOC = Research Institute of Oilseed Crops
RIFC = Research Institute of Fodder Crops

Report from France on *Brassica* genetic resources activities

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Review of data in the central database and planning of further data transfer

The French INRA-ENSA collection has now been completely transferred to Bras-EDB. It consists mainly of French *Brassica oleracea* landraces collected over the period 1980-1990. No further collecting activities have been undertaken.

A new project is being established in France under the leadership of G. Thomas, based on a *Brassica* French network. The main idea is to bring together the PGR activities of all partners (private and public) involved in vegetable *Brassica* PGR. A first joint action will be undertaken in 2002-2003 in order to establish a database, including the collection mentioned above but enlarged to include original *Brassica* landraces and populations conserved by each partner of the network (breeders). The objective is to centralize data and to share regeneration and evaluation activities among the different partners.

Inclusion of characterization and evaluation data in the database

- The French INRA-ENSA collection is completely described for passport data. For minimum characterization, on the basis of the minimum descriptor list, 44 accessions are characterized (24 *B. oleracea* var. *capitata* and 20 *B. oleracea* var. *botrytis*).
- For evaluation, the collection is now described for *Plasmodiophora brassicae* resistance (clubroot*)* (all *Brassica oleracea* accessions).
- A specific sample of *Brassica oleracea* populations (184 populations, including 96 *B. oleracea* var. *acephala*, 45 *B. oleracea* var. *capitata* and 43 *B. oleracea* var. *botrytis*) has been particularly studied with:
  - RAPD markers (100)
  - morphological markers (20 criteria measured)
  - *P. brassicae* resistance
  - S alleles (incompatibility).

The French network (see database) will undertake specific actions in 2002-2003 including evaluation for disease resistance (*Plasmodiophora* and *Xanthomonas*) and for the minimum IPRGRI descriptor list (one location; 2 x 25 plants).

As part of the GEN RES *Brassica* project, characterization is ongoing (≅ 40 accessions per year).

Safety-duplication and regeneration

Safety-duplication

In 2001, safety-duplication samples were prepared for part of the INRA-ENSA collection. The whole collection should be prepared in 2002.

Regeneration

Since 1998-1999, regeneration of accessions for the INRA-ENSA collection has been systematically undertaken. About 20 accessions per year have been regenerated, concentrating on those with the poorest germination levels.

This regeneration programme will also be included as part of the activities of the French network for each partner, and during 2002-2003 should result in 80-90 more regenerations.

Status report update on the *Brassica* collections in German genebanks

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In comparison to the number of accessions maintained in German genebanks six years ago, there has been a decrease of approximately 500 (4708 in 1996 - see Dehmer and Hammer 1997 - vs. 4212 in 2002). These changes can be attributed mainly to rationalization of the collection at the Malchow branch station of the IPK Genebank (1451 vs. 1048), whereas only minor changes took place in the IPK Genebank at Gatersleben (2045 vs. 1975) and in the BAZ Gene Bank at Braunschweig (1212 vs. 1189) (see also Table 1). The decrease in accessions at Malchow was mainly due to a concentration of the fodder and oil types there, with the vegetable brassicas being transferred to Gatersleben, as well as to a significant reduction in *B. napus* breeding lines conserved there in earlier times.

Nevertheless, the six economically important *Brassica* species (*B. carinata*, *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea* and *B. rapa*) still constitute more than 90% of the entire genus collection in Germany, and there were no changes in the location of the largest collections of the respective species (BAZ: *B. carinata* and *B. nigra*; IPK/Gatersleben: *B. juncea*, *B. oleracea* and *B. rapa*; IPK/Malchow: *B. napus*; Table 1). In addition to these, some progenies from interspecific crosses are an additional part of the Malchow collection, while 25 other (mainly wild) species of the genus *Brassica* are maintained at Gatersleben (taxonomic classification according to Gladis and Hammer 1990). Here, one species was lost since 1996 (*B. scopulorum* (Pomel) Batt.) and the entries from two were transferred to other species (*B. alboglabra* Bailey to *B. oleracea* L. and *B. perviridis* Bailey to *B. rapa* L. em. Metzg.). *B. barrelieri* (L.) Janka, *B. bivoniana* Mazzola & Raimondo, *B. desnottesii* Emb. et Maire, *B. drepanensis* (Gar.) Dam., *B. insularis* Moris, *B. maurorum* Dur., *B. montana* Pourr., *B. spinescens* Pomel and *B. taurica* (Tzvel.) Tzvel. were included as new species, with the status/identity of '*B. inisma*', obtained from a botanical garden, being unclear.

Concerning the active acquisition of new material, IPK Gatersleben collected almost 100 additional entries in mainly European countries between 1996 and 1999 (Table 2), either belonging to *B. napus*, *B. oleracea* or *B. rapa* or not determined yet.

In the near future, the transfer of the BAZ *Brassica* collection to the IPK as a result of the merging of the German *ex situ* genebanks for cultivated plants will very probably lead to further rationalization of the respective plant genetic resources of this genus, mainly due to a reduction of duplicated accessions.

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Table 1. Numbers of *Brassica* accessions in German genebanks

	Total		DEUBGRC		DEUIPK		DEUIPKM	
***Brassica* species**	**1996**	**2002**	**1996**	**2002**	**1996**	**2002**	**1996**	**2002**
B. alboglabra Bailey	15	-	-	-	13	(*)	2	(*)
B. balearica Pers.	1	3	-	-	1	3	-	-
B. barrelieri (L.) Janka		3	-	-	-	3	-	-
B. bivoniana Mazzola and Raimondo		1	-	-	-	1	-	-
B. bourgeaui (Webb in Christ) Kuntze	2	1	-	-	2	1	-	-
B. carinata A. Br.	155	152	115	115	37	37	3	-
B. cretica Lam.	3	6	-	-	3	6	-	-
B. desnottesii Emb. et Maire		1	-	-	-	1	-	-
B. drepanensis (Caruel) Damanti		3	-	-	-	3	-	-
B. elongata Ehrh.	8	7	-	-	7	7	1	-
B. fruticulosa Cyr.	10	11	-	-	9	11	1	-
B. gravinae Ten.	5	5	-	-	5	5	-	-
B. incana Ten.	3	4	-	-	3	4	-	-
B. inisma (?)		1(?)	-	-	-	1	-	-
B. insularis Moris		6	-	-	-	6	-	-
B. juncea (L.) Czern.	287	261	93	94	131	103	63	64
B. macrocarpa Guss.	7	5	-	-	7	5	-	-
B. maurorum Dur.		4	-	-	-	4	-	-
B. montana Pourr.		2	-	-	-	2	-	-
B. napus L.	1343	1066	314	319	198	104	831	643
B. narinosa Bailey	5	3	-	-	1	3	4	-
B. nigra (L.) Koch	177	157	97	77	46	17	34	63
B. oleracea L.	1587	1413	393	407	950	935	244	71
B. oxyrrhina (Coss.) Coss.	4	1	-	-	2	1	2	-
B. perviridis Bailey	2	-	-	-	2	(**)	-	-
B. rapa L. em. Metzg.	860	834	200	176	450	467	210	191
B. repanda DC.	2	4	-	-	2	4	-	-
B. rupestris Raf.	6	6	-	-	6	6	-	-
B. scopulorum (Pomel) Batt.	1	-	-	-	1	-	-	-
B. souliei (Batt.) Batt.	4	2	-	-	3	2	1	-
B. spinescens Pomel		1	-	-	-	1	-	-
B. taurica (Tzvel.) Tzvel.		1	-	-	-	1	-	-
B. tournefortii Gouan	10	14	-	-	10	14	-	-
B. villosa Bivona-Bernardi	6	2	-	-	6	2	-	-
B. sp. x sp.		15	-	-	-	-	-	15
B. sp.	205	217	-	1	150	215	55	1
Total	**4708**	**4212**	**1212**	**1189**	**2045**	**1975**	**1451**	**1048**

(*) see *B. oleracea;* (**) see *B. rapa*

Data mainly from IPK and Bras-EDB (Version 2001)

DEUBGRC = Federal Centre for Breeding Research on Cultivated Plants (BAZ), Braunschweig, Germany

DEUIPK = Institute of Plant Genetics and Crop Plant Research (IPK)-Genebank, Gatersleben, Germany

DEUIPKM = IPK–Genebank / Malchow External Branch Station, Malchow/Poel, Germany

Table 2. New *Brassica* accessions at the IPK Genebank at Gatersleben since 1996 (continued from Gladis and Hammer 1994 and Dehmer and Hammer 1997)

Year	Collecting mission to	No. of accessions	***Brassica* species**
1996		**15**	
	Spain/Balearic Islands	1	*B.* sp.
	Italy	6	*B. oleracea* (1), *B. rapa* (5)
	Croatia	8	*B. oleracea* (2), *B. napus* (1), *B. rapa* (2), *B.* sp. (3)
1997		**31**	
	China/Tibet	8	*B.* sp.
	Croatia	13	*B. rapa* (1), *B.* sp. (12)
	Italy	8	*B. oleracea* (2), *B.* sp. (6)
	Kazakhstan	2	*B.* sp.
1998		**18**	
	Korea	1	*B.* sp.
	Bulgaria	12	*B.* sp.
	Italy	5	*B.* sp.
1999		**32**	
	Spain	32	*B.* sp.

Brassica regeneration activities carried out in Italy

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Introduction

In Italy *Brassica* germplasm is mainly conserved by the national genebank located in Bari at the Istituto del Germoplasma (IDG), belonging to the National Research Council (CNR), and by five other institutions (three in Sicily, one in Marche and one in Umbria). The majority of *Brassica* germplasm is conserved in Sicily by the University of Catania (Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, DOFATA) and by the University of Palermo (Botanical garden (BG) and Dipartimento di Agronomia, Coltivazione Erbacee e Pedologia (ACEP)). The former holds an active collection of about 460 accessions, mainly landraces and wild species, while the latter holds a collection of wild species and the related herbarium.

Regeneration activities are essential to utilize the biological materials conserved in view not only to store them over time but above all to use them for characterization and evaluation activities and for safety-duplication by other institutions or genebanks. Regeneration of *Brassica* species, which are often self-incompatible, requires specific management for controlled pollination, achieved both by spatial isolation in the field or by the use of isolation chambers, in order to maintain the original genepool.

Of about 1500 *Brassica* accessions conserved in Italy by the above-mentioned institutions, only about 7% have been regenerated recently (Table 1). We describe here the regeneration activities carried out at DOFATA to support the activities dealing with characterization and evaluation and provide all the conditions required for exploiting some types and landraces of interest for vegetable diversification. These activities include in particular those carried out at DOFATA in the last two years dealing with specific tasks of projects funded by the Italian Agriculture Ministry and the European Union. The former is a project related to evaluation and to genetic improvement of violet cauliflower landraces whereas the latter is the GEN RES CT99 109-112 project in which DOFATA has contributed to the regeneration of a European *Brassica* core collection.

Table 1. *Brassica* accessions conserved and regenerated in Italian institutions

	No. of accessions		
Institution[1]	**Conserved**	**Regenerated**	**Method[2]**
ACEP	8	8	op
BG	400	-	-
DBVBA	17	3	op
DOFATA	457	48	cp
IDG	629	37	cp
ISPORT	50	18	op
Total	**1561**	**114**	

[1] ACEP = Dipartimento di Agronomia, Coltivazione Erbacee e Pedologia, University of Palermo
BG = Botanical Garden, University of Palermo
DBVBA = Dipartimento di Biologia Vegetale e Biotecnologie Agro-ambientali, University of Perugia
DOFATA = Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, University of Catania
IDG = Istituto del Germoplasma, CNR, Bari
ISPORT = Istituto Sperimentale per l'Orticoltura, Monsanpolo del Tronto, Ascoli Piceno

[2] op = open-pollinated; cp =controlled pollination

Methodology

The protocol to regenerate accessions at DOFATA begins by by sowing seeds in cellular trays in a greenhouse and transplanting plantlets (3^{rd}-4^{th} leaf stage) into 10-litre pots filled with a peat/sand substrate (1:1 in volume). Plants are usually grown in the open till they reach the flowering stage and then moved to the isolation chambers either in a cold greenhouse or in the field. In some cases plants are transplanted directly into the field and we then use isolation chambers covered by nets to protect plants. Usually about 40-50 plants per accession are put into each chamber for a variable period of time depending on the variation in the flowering time uniformity of the plants grown for each accession.

As pollinators, flesh flies (*Sarcophaga carnaria*) are used. These are grown at the institute, starting with larvae bought in fishing tackle shops. Each week about 1000-2000 larvae are grown in one-litre containers filled with peat and covered by a net till the adult stage is reached. Metamorphosis occurs when the temperature is between 13°C and 28°C, depending on the season; on average, 70-80% of larvae reach the adult stage. Adult flesh flies are released into the isolation chambers when the plants reach the flowering stage.

During the presence of the flies in the chambers no chemical product is used to control pests and diseases to avoid killing the pollinators. Sometimes a protein-rich commercial product (Bumital®) is sprayed in order to prolong the life of the flies in the chambers, especially when there are few flowering plants to feed them. At the end of the flowering stage the plants are moved from the chambers to the field to complete the fruit ripening stage.

Results

The activities carried out have so far allowed the regeneration of 48 accessions of *B. oleracea* var. *botrytis*, *B. oleracea* var. *acephala* and some *Brassica* wild species widespread in Sicily (Fig. 1).

Fig. 1. Some stages of *Brassica* regeneration activities carried out at DOFATA.

Metamorphosis took place at 8 and 18 days at 28°C and 13°C respectively (Fig. 2). This aspect is very important to synchronize the availability of adult flesh flies with plant flowering in order to regenerate all the plants utilized for each accession at the right time. The mean seed yield obtained per plant, for the accessions regenerated, was 28.4 ± 18.4 g for cauliflower, 21.8 ± 15.5 g for kale and 8.8 ± 2.6 g for some wild *Brassica* species. The protocol used is simpler and more efficient than those based on the use of honey bees (*Apis mellifera*) and bumble bee (*Bombus terrestris*) colonies which are much more expensive and not very suitable for the small isolation chambers we use.

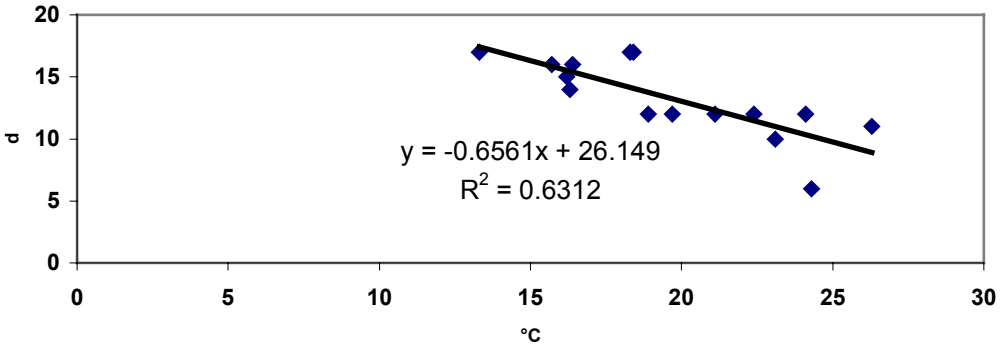

Fig. 2. Correlation between temperature and metamorphosis timing.

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Status of *Brassica* germplasm collections in Poland

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Collections

The conservation work on species of the genus *Brassica* within the framework of the National Programme for Plant Genetic Resources Conservation is mainly conducted at two institutions: the Borowo Plant Breeding Station, where field crops are multiplied and evaluated, and the Research Institute of Vegetable Crops (Kotlińska 1997). The *Brassica* collection consists of 1265 accessions (Table 1).

Table 1. Status of *Brassica* germplasm in the National Programme for Plant Genetic Resources

Name	No. of accessions
Broccoli	16
Cabbage	284
Cauliflower	164
Fodder Cabbage	19
Fodder Rutabaga	17
Fodder Turnip	3.
Kale	6
Kohlrabi	13
Mustard	19
Radish	15
Rape	517
Rapistrum	43
Rutabaga	17
Small radish	61
Turnip	36
Watercress	5
Other	30
Total	**1265**

Collecting missions

Collecting missions are organized systematically to regions rich in indigenous materials in Poland and neighbouring countries. Kotlińska (1997) summarized the results of expeditions carried out from 1991 to 1995, during which 30 *Brassic*a accessions were collected. Table 2 provides data from missions carried out over the period 1996-2001 in Poland, Ukraine, Moldova, Slovakia and the Czech Republic. A total of 112 accessions were collected.

Table 2. *Brassica* accessions collected during missions carried out between 1996 and 2001

Species	Country of origin	No. of collected accessions
Brassica campestris	1 POL	1
Brassica napus napobrassica	12 POL; 6 UKR; 2 SVK; 1 CZE	19
Brassica nigra	11 UKR	11
Brassica oleraceae	21 POL; 17 UKR; 2 MDA	40
Brassica rapa	1 POL; 8 UKR	9
Crambe maritima	2 UKR	2
Sinapis alba	16 POL; 2 UKR	18
Raphanus raphanistrum	2 UKR	2
Raphanus sativus	4 POL; 4 MDA; 2 UKR	10
Total		112

Reference

Kotlińska, T. 1997. Status of the *Brassica* germplasm in the collection of vegetable crops in Poland. Pp. 50-53 *in* 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, Rome, Italy.

Status of the *Brassica* collection in Russia

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The collection

The *Brassica* collection in Russia consists of 6603 accessions belonging to 9 species. A review of the current status of the *Brassica* collection is given in Table 1. The collection includes wild species (2n=18), landraces, old and advanced cultivars, F_1 hybrids and breeding materials, mainly sterile and self-incompatible lines.

The Russian *Brassica* collection was established in 1923 and the genebank now contains genetic resources from 75 countries. Unfortunately the number of seed collecting expeditions has been reduced.

Table 1. Status of the VIR *Brassica* collection

Species	Subspecies/variety	No. of accessions	Status*			
			LR	CV		BR / H_1
				Old	Advanced	
B. *campestris* L.	*oleifera* DC	327	118	23	92	94
B. *carinata* A.Braun		49	40		3	6
B. *juncea* (L.) Czern.	lettuce	27	27			
	oil	1110	838	53	125	94
B. *napus* L.	*oleifera* Metzg.	1012	130	120	479	283
	biennis (DC) Reichb.	6		6		
	rapifera Metzg.	247	51	169	27	
B. *nigra* (L.) Koch		40	19	4	5	12
B. *oleracea* L.	*acephala*	141	30	53	45	13
	alboglabra	7	7			
	botrytis	698	27	159	436	76
	capitata (white group)	1175	235	249	343	348
	capitata (red group)	171	3	53	91	24
	costata	34	34			
	gemmifera	167		17	49	101
	gongylodes	155	26	55	57	17
	italica	167	6	25	40	96
	sabauda	148	2	64	49	33
B. *rapa* L.	*chinensis*	109	57		44	8
	japonica	14	2		11	1
	rapa	384	30	300	40	14
	rapa f. *Komatsuna*	22	14		8	
	pekinensis	382	88		97	197
Wild		11	11			
Total		**6603**	**1795**	**1350**	**2041**	**1417**

* LR = landraces; CV = cultivars; BR = Breeding materials and F_1 hybrids

The Russian *Brassica* collection is divided into two parts: a permanent (basic) catalogue (76%) and a temporary catalogue. The permanent catalogue includes landraces and breeding cultivars with a sufficient quantity of seeds (more than 6000 seeds). All accessions of the permanent catalogue are documented for passport data. Passport data are stored in Excel files in the Vavilov Institute and they can also be found on VIR's homepage on the Internet (http://www.vir.nw.ru). The temporary catalogue includes the F_1 hybrids, breeding materials and samples with insufficient seeds (less than 6000 seeds). These latter cultivars need to be regenerated and they will then be included in the permanent catalogue. Passport data for these accessions are stored in our documents.

Characterization and evaluation

All accessions of the *Brassica* collections have been characterized and evaluated for many morphological, biological and agronomic traits at several experimental stations, which are located within different ecological/geographical zones of Russia. The accessions have been studied using the same standardized research methods for three years. The main data include 40-43 characters of the vegetable crops *Brassica oleracea*, *B. rapa* and *B. juncea*, and 18-23 characters of oilseeds *Brassica.* Evaluation data are stored in summary journals and in separate computerized database files (e.g. biochemical data, data of the electrophoretic analysis of seed proteins, data of *winter cauliflower*, *Chinese cabbage, pak-choi*, etc.).

Some of the accessions have been tested for resistance to cold, heat, drought, salt and diseases (downy mildew, yellow, black rot and clubroot) in natural and artificial infectious conditions. The results of these tests are given in separate non-computerized data.

The Vavilov Institute regularly publishes *Delectus seminum* listing all accessions available for exchange. Evaluation data can be provided to partners for international projects (e.g. creation of a core collection). It is also possible to prepare the evaluation data according to minimum descriptors.

Utilization

The Vavilov Institute publishes the evaluation catalogues of studied accessions for breeders. Every year 550 seed samples of the collection of vegetable *Brassica* and 350 samples of oilseed *Brassica* are distributed for utilization to breeding institutes/centres and companies including 300 samples sent abroad.

Safety-duplication and storage

All *Brassica* collections are located in St. Petersburg in aluminium boxes at room temperature. From 1965 to 1995, 61% of accessions in the *Brassica* base collections were duplicated in St. Petersburg at 4°C under vacuum in glass jars (3000 seeds) (Table 2). Since 1996, 34% of accessionshave been duplicated in refrigerators at a temperature of –10°C for long-term storage.

Since 1975, 57% of accessions of the *Brassica* base collections have been duplicated in the National Seed Storage at Kuban experimental station at 4°C in glass jars (6000 seeds per accession). The seeds are dried to a moisture content of about 6%.

Also 8% of the accessions of the *Brassica* base collections have been duplicated in VIR Genebank in refrigerators for long-term storage at –10°C in laminated aluminium packages (6000 seeds per accession). The seeds have been dried.

A new cold storage has just been built in St. Petersburg. The working (active) collections will be stored at 4°C in laminated aluminium packages for short-term storage and all base collections of VIR will be duplicated at -10°C in laminated aluminium packages for long-term storage.

Regeneration

The accessions of *Brassica* collections are sown for regeneration every fourth–fifth year at eight experimental stations of VIR with the exception of the accessions of the "passive"[11] part of collections duplicated in long-term storage in St. Petersburg. The criterion for regeneration of accessions stored in the National Seed Storage is germination below 50%.

A few accessions are regenerated on isolated plots (number of plants 60-100, natural pollination by honey bees and flies). Many accessions are regenerated in isolation chambers and some accessions in plastic greenhouses (number of plants 30-40, pollination by

[11] The "passive" part of the collection includes accessions under study, and the value of which has not yet been established or confirmed

microfamilies of bees, flies and manual). Fifty percent of the accessions of the vegetable *Brassica* collection are regenerated during winter in southern Russia.

Table 2. Storage status of VIR *Brassica* collection

Species	Subspecies/variety	Passport data	Storage			
			Long-term	Short-term	NSS* (since 1975)	Cold (1965-1995)
B. campestris L.	*oleifera* DC	317		56	119	41
B. carinata A.Braun		49		15	38	28
B. juncea (L.) Czern.	lettuce	12		7	9	5
	oil	1102		104	866	857
B. napus L.	*oleifera* Metzg.	896		325	442	200
	biennis (DC) Reichb.	6		4	5	
	rapifera Metzg.	247	199		229	74
B. nigra (L.) Koch		36		9	24	18
B. oleracea L.	*acephala*	99	1	41	45	99
	alboglabra	4		1	1	
	botrytis	477	21	240	203	343
	capitata (white group)	741	24	473	367	716
	capitata (red group)	95		45	17	86
	costata	27	5	13	7	20
	gemmifera	23	1	4	3	20
	gongylodes	110	2	48	43	78
	italica	61	3	15	36	61
	sabauda	103		91	72	90
B. rapa L.	*chinensis*	45	2	39	27	40
	japonica	8		7	9	5
	rapa	384	143		221	158
	rapa f. *Komatsuna*	4		2	2	
	pekinensis	181	4	144	99	139
Wild		4		2	1	1
Total		**5031**	**405**	**1715**	**2885**	**3079**

* NSS = National Seed Storage

Research

Research activities dealing with resistance of *Brassica* to abiotic factors are currently in progress. Great attention is paid to prebreeding evaluation of the biochemical composition of the accessions (dry matter, sugar, oil, protein, acids, glucosinolates, vitamin C, carotene, including β-carotene and chlorophylls). Electrophoretic analysis of storage proteins of seeds is used for the study of the taxonomic similarity of accessions, evolution of cultivar groups and differences between them within *Brassica* crops.

The creation of new Russian hybrids of spring rape uses different systems of cytoplasmic male sterility (CMS).

Research on wild species

The collection includes wild species related to *Brassica* crops: *B. incana* Ten., *B. cretica* Lam., accessions of *B. sylvestris* L. from the Atlantic coast. The only wild species of *Brassica* (2n=18) located on the territory of former USSR is wild kale *Crimea* – endemic in Crimea. Its taxonomic position needs to be determined. According to morphological and biological traits and electrophoretic analysis of seed globulins, we think the population of wild kale *Crimea* to be a separate part of the *B. incana* population. The samples of this kale are resistant to low temperature and clubroot and possess valuable biochemical leaf composition.

We have noted morphological and biological similarities between kohlrabi and *B. incana* Ten., such as a similar 12S globulin content. Consequently we believe that kohlrabi originated from *B. incana* and cauliflower from *B. cretica.*

Identification of the accessions can be carried out by molecular methods.

Future activities

Future activities include the creation of the computerized evaluation database of all *Brassica* accessions and long-term storage of seeds in refrigerators at St. Petersburg.

Update for 2002

During 2002, the base collections of *Brassica oleracea* and leafy *B. rapa* at VIR have increased by 130 accessions, after these have been multiplied and studied. These are generally local and advanced breeding cultivars of *B. rapa* subsp. *pekinensis, chinensis, japonica* and *narinosa* from North and East China, Japan and Korea. These cultivars contain sources of high yield, early maturity, resistance to downy mildew and tolerance to clubroot. According to Japanese breeding companies, some of the Chinese cabbage accessions are resistant to clubroot. However, these cultivars and hybrids only show tolerance to clubroot in the area of St. Petersburg, where the strongest clubroot races can be found. Russian hybrids of Chinese cabbage with genetic clubroot resistance (obtained from a line of *B. rapa*) have recently been included in the VIR collection. The local Chinese samples of *B. rapa* subsp. *chinensis* and *pekinensis* are often used as autumn crops for leaf consumption and as spring crops for oilseed. Oil content was measured, ranging from 31.5 to 38.2%. The fatty acids profile was as follows:

Palmitic	1.6-2.8%
Stearic	0.5-1.2%
Oleic	12.5-19.1%
Linoleic	11.6-13.7%
Linolenic	8.5-10.1%
Arachidonic	0.6-0.9%
Eicosenoic	7.0-10.2%
Erucic	41.7-55.9%
Docosanic	1.2-2.0%

In addition, the base collection of *B. oleracea* was enriched with cultivars of winter cauliflower and white cabbage from former USSR and India. Accessions of the base collection were deposited in the VIR genebank refrigerators for long-term (400 accessions) and short-term storage (200 accessions).

The *Brassica carinata (*Braun) collection in Russia

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The Abyssinian mustard *Brassica carinata* (n=17, genome BBCC) is an endemic plant of the Ethiopian plateau, a natural cross between the wild *Brassica nigra* (n=8, genome BB) and forms of *Brassica oleracea* (n=9, genome CC). Its electrophoretic spectrum includes the spectrums of paternal forms.

The first accessions of Abyssinian mustard were included in the VIR collection in 1960. The collection now contains 49 entries (weed populations and cultivars) from Ethiopia, Kenya, Tanzania, India, Pakistan and Canada. The samples have been evaluated on the plots of VIR field station in the Krasnodar region. The accessions were late-maturing and have a seed yield of 60-300 g/m^2.

The Abyssinian mustard was researched as an oilseed crop. The material has generally exhibited some variation. Oil content varied from 22.9 to 39.7%. The fatty acids profile was as follows:

Palmitic	2.0-4.1%
Stearic	0.9-1.1%
Oleic	5.9-19.6%
Linoleic	14.1-18.2%
Linolenic	12.8-18.9%
Eicosenoic	6.7-9.4%
Erucic	32.1-51.2%

The *B. carinata* seed contained high concentrations of allyl-glucosinolates.

B. carinata plants are known to be the most salt- and heat-tolerant of the Brassicaceae family. All cultivars of Abyssinian mustard were free from the mustard aphid *Lipaphis erysimi* (Kalt.). This species is reported to be highly resistant to the pathogen fungi of black rot (*Xanthomonas campestris* pv. *campestris)* and black leg (*Leptosphaeria maculans).* The allohexaploid *Brassica* x *composita* (2n=54, genome AABBCC) was created in Russia by interspecific hybridization of lines of *B. carinata* and *B. rapa*. This hybrid is the donor of resistance to clubroot and black rot.

B. carinata is a potential source of disease and pest resistance and other useful traits for *Brassica* oilseed crop breeders.

Identification of *Brassica* species by storage seed proteins and evolution of the genus *Brassica*

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The use of storage seed proteins as molecular markers in addition to morphological traits allows a more efficient study of the genepools of many crops including *Brassica* sp. (Konarev 1993).

Brassica seeds contain two groups of storage proteins—albumins and globulins. The globulin fraction is more abundant. It comprises 12-14S proteins, which by analogy with similar protein species have been named cruciferins. A cruciferin protein, like other proteins referred to this class, is a complex oligomeric molecule consisting of several types of subunits. Each cruciferin subunit is a complex of acid (α) and basic (β) polypeptide chains crosslinked with disulfide bonds. It is encoded by a single gene and synthesized as the precursor form. Gene families coding for *cru1*, *cru2/3* and *cru4* subunits have been identified (Schwence *et al*. 1983; Sjodahl 1994).

Intra- and interspecific variability of cruciferins was studied by screening a large group of specimens stored in the VIR collection and representing the *Brassica* L. genus (wild species, old and advanced cultivars, lines, hybrids). Electrophoresis was carried out under dissociating conditions, i.e. in presence of SDS and ME which break down and restore disulfide bonds between polypeptides. The analysis of more than 25 000 individual genotypes has revealed multiple polypeptide complexes differing in their molecular weights and manifestation. All cruciferin polypeptide species that have been identified are numbered and presented as a reference (summarized) spectrum (Fig. 1). Such spectra have been built for every genome. In total, 29 polypeptide positions numbered from higher to lower electrophoretic mobility have been distinguished. We believe that this type of numbering is the most convenient and facilitates computer-assisted processing. The identified components correspond to acid and basic polypeptides, the boundary between them being marked by a 25 kDa marker protein chymotrypsinogen (Fig. 1). The components above the 25-kDa marker are acid polypeptides (α) corresponding to positions 16-29 of the spectrum, whereas those below the marker are basic proteins corresponding to positions 1-15. In our opinion, this structure of the spectrum reflects the differentiation of the proteins according to their biochemical features and to their structural organization. Cruciferin spectra of all three genomes contain many common components. However, there are polypeptides that predominate in most genotypes of a given genome. We ascribe the status of the principal polypeptides to them. They are underscored in Fig. 1.

Our data for the variability of cruciferin polypeptides in seeds of different species of the *Brassica* genus allow this storage protein to be used as a marker in the selection and phylogenetic analysis of *Brassica*.

The specific distribution of cruciferin components within each genome provides for the identification of inter-genome hybrids and natural amphidiploids, ascertaining their genomic attribution, and for determination of interrelationships between wild and cultivated species. Representatives of each genome have been found to feature definite polypeptide patterns. Thus, the cruciferin of all C-genomic *Brassica* species occupies component positions 4-13 and 16-25. Species that represent this genome lack the high molecular components 26-29 and basic components 14-15. In A-genomic species, to which all East-Asian *Brassica* species belong, specific components have been found along with those that are common to all three genomes (positions 3, 15, 26, 28). Components 10, 19 and 25 are relatively consistent in different East-Asian *Brassica* species. The total cruciferin spectrum of a B-genomic species

B. nigra L. comprises polypeptide variants migrating to positions 5, 9, 1, 12, 17, 19, 20, 21, 23, 25, 26, 27 and 29. In all genotypes the principal components at positions 5, 12, 29, 23 and 25 have been found. However the last two are scant.

Thus, the patterns of the electrophoretic spectrum of cruciferin may be used for the genomic attribution of a given specimen. For example, electrophoretic analysis of cruciferin from seeds of *B. alboglabra* L. confirmed its attribution to C-genomic but not to East-Asian *Brassica* species. The following *Brassica* species have been identified as Asian (A-genomic): *B. perviridis*, *B. nipposinica*, and *B. narinosa*. *B. insularis*, *B. elongata*, *B. macrocarpa*, *B. incana*, *B. balearica*, *B. sylvestris* (k-289 and k-48) and *B. cretica* have been attributed to C-genomic species. In our opinion, *B. integrifolia* and *B. fruticulosa* should be attributed to B-genomic species. The *B. oxyrrhina* species, which has n=9, as have C-genomic species, differs substantially from the latter in its protein pattern. No similarity in the 12S-globulin pattern has been found between this species and B- and C-genomic species. Thus, *B. oxyrrhina* represents a separate genomic type. It is designated as O-type in the literature. A similarity in polypeptide composition has been found between this species and *B. barrelieri*. Specific patterns of polypeptide components within a genome may help to identify inter-genomic hybrids. A confirmation of this possibility has been obtained in the analysis of natural amphidiploids of the mustard *B. juncea* L. (genome AB) and the Abyssinian mustard *B. carinata* (genome BC) whose electrophoretic spectra include the features of the spectra of their parental forms. We have revealed a similarity in polypeptide spectra between *B. juncea* L. and a specimen of *B. nigra* L. from the Chimkent area of Uzbekistan, which may be evidence of the contribution of *B. juncea* L. to the origin of *B. nigra* variants related to the above specimen. Analysis of the species interrelations is important not only for studies of systematics and phylogenetics of a given genus, but also for selection based on hybridization of remote species carried out in order to combine a complex of valuable traits within a single genotype.

On the basis of the composition of genetic variants of cruciferin, one can identify cultivars and hybrid populations, mark lines, or detect contaminations of hybrid seeds with inbred seeds. Cruciferin polypeptide composition provides for the identification of inbred lines provided that all their plants feature identical spectra. Control tests based on cruciferin spectrum analysis proved to be useful in the development of inbred strains of Asian *Brassica rapa* varieties. The lines kept at VIR for up to four inbreeding generations have been developed on the basis of 12 parental cultivars. Nine of these lines appeared morphologically homogeneous in the third generation of inbreeding, which was confirmed by electrophoretic analysis of seed collections showing that these strains had similar spectra. It is important that all these strains featured individual electrophoretic patterns providing for their reliable identification.

We used two approaches to assess the composition of *Brassica* cultivars. The first approach consists of using a polypeptide complex or spectrum type as a protein formula. In this way we have identified 50 specimens of East-Asian *Brassica rapa* varieties. An analysis of a wide variety of vegetable *Brassica* cultivars has revealed that most cruciferin components differ in their manifestation. A more common manifestation may result from the superposition of free and bound polypeptides and polypeptides from different subunits. Therefore, the cultivars with similar spectra may differ in manifestations of their separate components. To account for that, we recorded each component separately and indicated the rate of its occurrence (%) within a given specimen. By using this approach, we have identified 130 cultivars of the most agriculturally important *Brassica* crops, i.e. white cabbage, cauliflower and kohlrabi. Among these *Brassica* crops, the greatest inter-cultivar and intra-cultivar variability is found in kohlrabi and the lowest in cauliflower. The polymorphism of storage globulin is known to depend on the genetic variability of the parental plants (sources of origin), the agricultural area, the mode of pollination and the rate of selection. Since all agricultural *Brassica oleracea* crops are cross-fertilizing and their cultivation areas are very

broad, the cause of the pronounced polymorphism of cruciferin should be sought for in their origin and their selection history. Kohlrabi has been subject to less breeding selection than white cabbage or cauliflower. Thus, it is most likely that kohlrabi originated from a highly polymorphic ancestor.

We have studied cruciferin composition by two-dimensional electrophoresis and determined polypeptide pairs that compose separate subunits. After a thorough comparison of our data with those available in the literature, it has been found that subunits of the *cru1* gene family are present only in A-genomic species (polypeptide pair 28-12) and B-genomic species (27-12), and absent in C-genomic species. It should be noted that polypeptide 12 is also present in C-genomic species but is a component of the *cru2/3* subunit. Subunit polypeptide pairs encoded by gene families' *cru2/3* and *cru4* are identified in all three genomes. However, in cruciferin molecules of the B-genome and, especially, C-genome, we have identified additional *cru2/2* subunits. This is in agreement with data for the role of duplication in the storage globulin family. It is known from the literature that the evolution of 12S-globulins occurred in two stages (Sjodahl *et al.* 1991). The ancestral cruciferin gene was duplicated and gave rise to parental forms of the modern *cru1* gene and the common parental gene for gene families *cru2/3* and *cru4*. Since these events occurred at the early stages of Brassicaceae evolution, they are directly associated with the divergence of the species of this family. Possibly, the A-genomic species that have subunits of all three families are more ancient, whereas the C-genomic species are the youngest from an evolutionary perspective.

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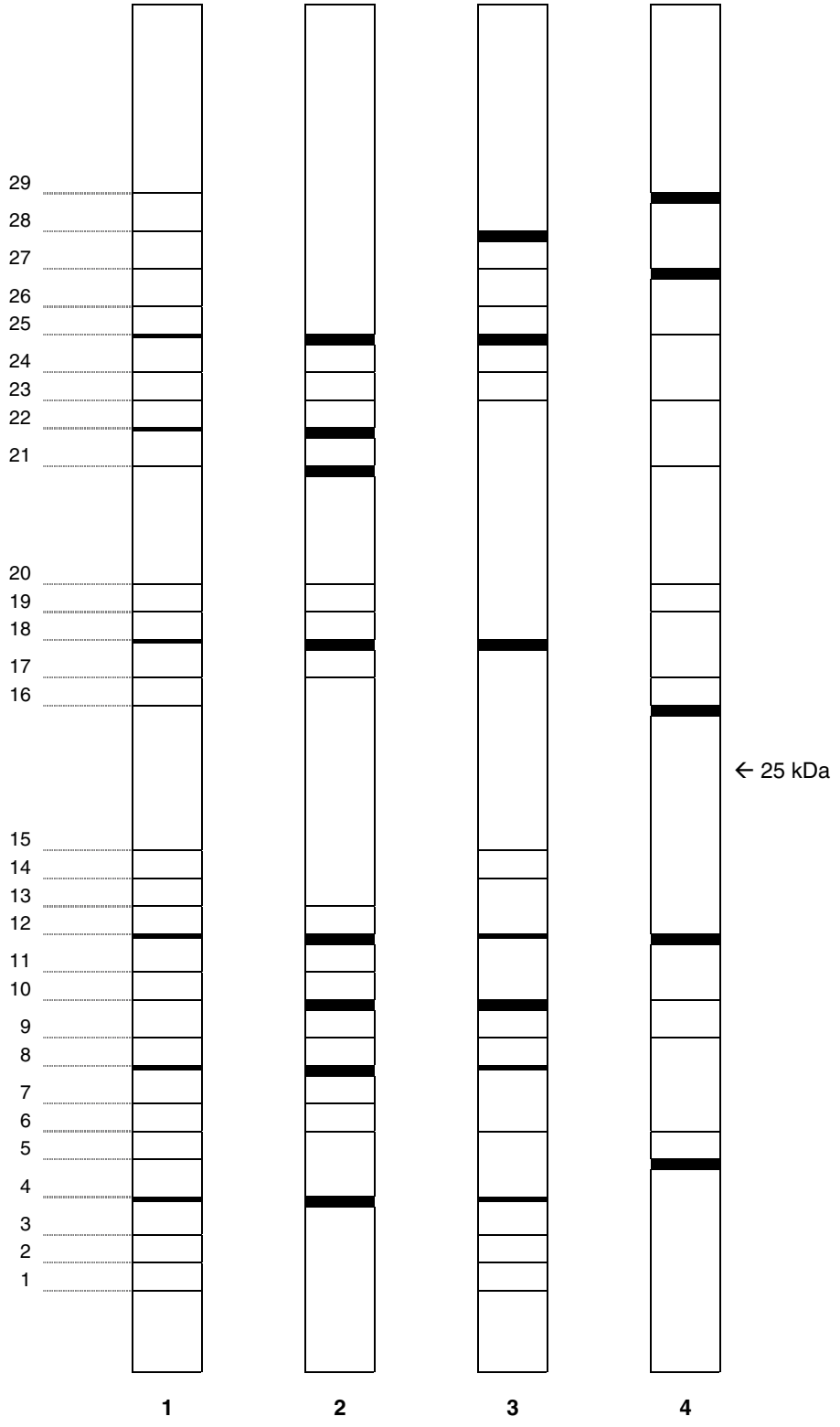

Fig. 1. Summarizing spectrum of cruciferin (1), spectrum of genome species (2), genome species (3) and genome species (4) of genus *Brassica* L.

Old local Russian cultivars of white cabbage

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The collection of *Brassica oleracea capitata alba* held at VIR consists of 1197 accessions, including 763 accessions in the permanent (basic) catalogue. These include 115 local Russian cultivars which have been known since the 18th century. Their origin and distribution areas are located near old commercial centres.

These cultivars were the sources of a few old breeding and advanced cultivars of Russian cabbage. Some of the local cultivars still retain their economic value in several regions of the country (e.g. Siberia and coast of Lake Ladojskoye), owing to their excellent agronomic traits.

The main ecogeographic groups of cultivars are presented below.

Cultivar type **'Saburovka'** includes local medium-late cultivars from the central zone of the European part of Russia. They do not require fertile soil and are highly tolerant to clubroot.

Type **'Savinskaya'** includes medium-late and late cultivars from the southeastern European part of Russia. They have a high level of resistance to heat, a high yield and long storage capacity. This type of cultivar possesses sources of resistance to clubroot, yellows and black rot. The heads of these cultivars are round-flat, large and white, with medium density, suitable for sauerkraut. The plants are very vigorous. These cultivars originate from 'Penca' and 'Veernaya' (Fan) *Brassica oleracea costata* and are distantly related to 'Braunsweiger'.

The Central-Russian group of cultivars **'Jurievetskaya'** and **'Moscovskaya pozdniaya'** were created in the Moscow area in a continental temperate humid climate on fertile soil. These cultivars are related to 'Saburovka'.

The late and medium-late cultivars are high-yielding (head weight 4-6 kg) and tolerant to clubroot, but they require fertile soil and humidity and are not resistant to frost. Heads are bright white, round and round-flat, solid and with extremely good taste. These cultivars contain a high percentage of dry matter, sugar, medium percentage of ascorbic acid and other acids, and are therefore suitable for the best quality of sauerkraut. Storage is possible for four months only.

The North-Russian group of cultivars was created in the St. Petersburg area. The old local cultivars of southwestern Europe and northern Russia obviously have a common origin.

The cultivars are slightly to moderately resistant to heat, slightly resistant to leaf and head diseases, but highly tolerant of clubroot.

The medium-early cultivars **'Kaporka'** have round or round-flat heads, small and very small, sometimes dwarf, with a slightly bitter taste and good keeping ability. The medium term and fairly late cultivars **'Valvatievskaya'** and **'Ladojskaya'** do not require fertile soil, are resistant to clubroot and produce high yields. Heads are large, flat and round-flat with medium density.

The old local cultivars of Siberian types **'Zaviletskaya'** and **'Mahrovolistnaya'** are closer to type 'Veernaya' *B. oleracea costata* than other local cultivars. They are medium-late, frost-resistant, with white round thick heads of medium size. The plants of type 'Mahrovolistnaya' are strongly colored by anthocyans.

According to the study of morphological and agronomic traits and electrophoretic analysis of 12S-globulins of seeds, the old Russian local cultivars are populations including 2-4 related biotypes. The comparison of 5-8 reproductions with the original seeds of many accessions showed that the genetic composition of these populations changed very little and genetic erosion was very rare.

The primary genetic material of white cabbage arrived in Russia 4-5 centuries ago from southwestern Europe and Asia Minor via Crimea (first wave), from Central Europe (second wave) and then from western Europe. Therefore the diversity within Russian local cultivars is large because they came from various and distant genepools.

The old Russian local cultivars can provide a potential source of genes and be used in cabbage breeding programmes.

Report on the Brassicaceae collection in Yugoslavia

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Status of the Yugoslav Brassicaceae collection and data in the Central Brassica *Database (Bras-EDB)*

During the last decade, much attention has been paid to the preservation of genetic resources of the Brassicaceae family, with the aim of preserving the genetic resources of Yugoslavia and establishing a Yugoslav Plant Gene Bank (BBGJ). Fifty-one genera of the Brassicaceae family have been described in Yugoslavia (Jovanović-Dunjić *et al.* 1972). From the *Brassica* genus, *B. elongata* has been described on Fruska Gora Mountain, near Belgrade, and in southern Serbia. *Brassica polymorpha* has been described as critically endangered and is distributed on Titel Hill and Fruska Gora Mountain. *Brassica nigra* is spread throughout Serbia. *Brassica oleracea, B. napus* and *B. rapa* are cultivated in almost all regions of Yugoslavia.

The national collection of cruciferous plants in Yugoslavia is steadily increasing. At the moment, it consists of 352 accessions of mostly *Brassica oleracea* varieties (Table 1) held at the Centre for Vegetable Crops in Smederevska Palanka (223 accessions) and at the Institute of Field and Vegetable Crops in Novi Sad (129 accessions).

Table 1. Collection of Brassicaceae in Yugoslavia

Species	No. of accessions
Brassica oleracea var. *capitata*	210
Brassica oleracea var. *botrytis*	5
Brassica oleracea var. *italica*	4
Brassica oleracea var. *gemmifera*	3
Brassica oleracea var *acephala*	2
Brassica rapa pekinensis	2
Brassica napus	82
Brassica oleracea var. *gongylodes*	2
Raphanus sativus var. *majori*	8
Raphanus sativus var. *radicula*	33
Alyssum markgrafii	1
Total	**352**

Collecting of old domestic populations of *Brassica* vegetables has a major influence on the breeding programme development. Most of these are cabbages (*B. oleracea* var. *capitata*). Some of these genotypes show increased tolerance to some diseases and pests and their use in breeding programmes is of great importance (Sretenović-Rajičić *et al.* 2000a, 2000b).

Passport data for 182 accessions from our collection were sent to the Bras-EDB.

Inclusion of characterization and evaluation data in the database

Characterization according to the multi-crop passport descriptor list developed by IPGRI is in progress. Collecting of passport data for all accessions is now in its terminal phase and these data will be transferred to the Bras-EDB once they are completed.

Characterization and evaluation of morphological and agronomic traits are in progress. So far data have been recorded for about 25% of our collection. This activity is time- and space-consuming and therefore every year plans are made to decide which accessions could

be included in it. Evaluation and characterization data will be computerized. Once all data are collected and completed, they will be available for further inclusion in the Bras-EDB.

Review of methodologies for safety-duplication and regeneration

Safety-duplication collection of our accessions practically does not exist yet. For the purposes of the BBGJ collection, a project for the creation of safety-duplication collections within Yugoslavia has been developed. According to this year's plans, 15 accessions from the Brassicaceae collection (domestic populations of cabbage) have been included in this programme.

A major objective of safety-duplicating the national collection is to preserve samples from accidents such as the flood in 1999, which led to severe damage of some of the samples included in the regeneration process in the glasshouse or conserved in the genebank chamber, situated in the basement.

Regeneration protocols have been established for most of our samples and data sent to NGB (Table 3).

In situ *conservation actions*

There are 223 species from the Brassicaceae family described in Yugoslavia (194 in Serbia and 148 in Montenegro). Some are endemic and endangered in our country and their conservation and collection is a very important task. For example, *Draba bertiscea* is a new species known from a single mountain locality on Prokletije, Montenegro (Lakušić and Stevanović 1995). Unfortunately, except for *Alyssum markgrafii*, which appears to be a hyper-accumulator species for nickel (Obratov *et al.* 1994), we have not collected or regenerated significant seed samples of all the valuable species belonging to this group. In the last few years areas where cruciferous species have been located have been mapped, which will contribute to further conservation, collecting and study (Stevanović *et al*. 1994, 1996). Table 2 lists the most endangered species in Serbia and species of international importance according to Stevanović *et al*. (1995) and Stevanović (1999).

Table 2. Most endangered species in Serbia (Yugoslavia) and internationally important species

Most endangered species
Alyssum linifolium
Hymenolobus procumbens
Cardamine trifolia
Chorisphora tenella
Erysimum marshalianum
Crambe tataria
Sisimbrium polymorphum
Barbarea lepuznica
Fibigia clypeata
Draba nemorosa
Draba siliquosa
Arabis bryoides

Species of international importance
Alyssum markgrafii O.E. Schulz
Alyssum montanum L. subsp. *serbicum* Novák
Berteroa gintlii Rohlena
Biscutella laevigata L. subsp. *montenegrina* Rohl.
Bornmuellera dieckii Degen
Cardamine pancicii Hayek
Draba bertiscea D. Lakušić and V. Stevanović
Draba korabensis Kummerle and Degen
Lunaria telekiana Ják.
Thlaspi bellidifolium Griseb.

Table 3. Regeneration protocols in Smederevska Palanka, Yugoslav Genebank (BBGJ)

Subtype	late	Early	midseason	midseason	midseason
Culton	white and red cabbage	white cabbage	radish	winter radish	kale
Species	*B. oleracea* var. *capitata*	*B. oleracea* var. *capitata*	*Raphanus sativus* var. *radicula*	*Raphanus sativus* var. *majori*	*B. oleracea* var. *acephala*
Criteria for regeneration	germination <75%	germination <75%	germination <70%	germination <70%	germination <75%
Sowing	June, open field, 18-20C	July, open field, 20-22°C	March, open field, 5-8°C	July, open field, 20-22°C	May, open field, 18°C
			thining out twice	thining out, July/August	
Planting	July/August, open field	August/Sept., open field			June/July, open field
No of plants	200	200	200	200	200
Distance (cm) in/between rows	50–60 / 70	40–50 / 50	20 / 50	30– 40 / 70	40 / 60
Off field					
Vernalization / overwintering	greenhouse, 4-10°C, 10 weeks	greenhouse, 4-10°C, 10 weeks		storage in the field	earthing up plants in the open field
2nd year in field				planting in the open field in March	soil removed from plants in March
Isolation	green house	green house	distance over 2000 m	distance over 2000 m	distance over 2000 m
Flowering time					
Pollination	honey bees	honey bees	honey bees	honey bees	honey bees
Seed harvest	several times	several times	once	once	twice
Post harvest treatment	gentle threshing	gentle threshing	drying, threshing	drying, threshing	gentle threshing
Disease / treatment	*Peronospora parasitica, Meligethes aeneus, Euridema* spp. / chemical treatment	*Peronospora parasitica, Meligethes aeneus, Euridema* spp. / chemical treatment	*Meligethes aeneus, Halticinae* spp. / chemical treatment	*Meligethes aeneus, Halticinae* spp. / chemical treatment	*Meligethes aeneus, Euridema* spp. / chemical treatment
Additional comments			annual plant		
Storage in genebank	4-8°C 50% air humidity	4-8°C 50% air humidity	4-8°C 50% humidity	4-8°C 50% humidity	4-8°C 50% air humidity
References	Miladinović *et al.* (1997)	Miladinović *et al.* (1997)	Miladinović *et al.* (1997)	Miladinović *et al.* (1997)	Miladinović *et al.* (1997)

Strengths and weaknesses in genetic resources work

One of the main aspects of our work is to include wild Brassicaceae species in our collection as much as possible. One of the major problems is funding, as well as regeneration, characterization and evaluation of the samples, which are time- and space-consuming.

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PART III. PRESENTED PAPERS - GEN RES SESSION

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The EU GEN RES CT99 109-112 project *Brassica*, including *B. carinata*

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Introduction

The GEN RES CT99 109-112 project "*Brassica* collections for broadening agricultural use, including characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop" started officially on 1 January 2000 and the research will be conducted over a 4-year period. The project, which is co-financed by the European Commission, Council Regulation (EC) No 1467/94, is a fusion of two separate proposals, namely GENRES 109 "*Brassica* collections for broadening agricultural use" and GENRES 112 "Characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop".

It aims to conserve, document, characterize, evaluate and rationalize European collections of four important *Brassica* species and will contribute to a better knowledge of the genetic resources of these important *Brassica* species and improve the utilization of the genepools in Europe by plant breeders and growers. The project is engaged in the following four *Brassica* species:

- *B. oleracea:* cole crops, e.g. kales, cabbages, cauliflower, broccoli, kohlrabi, couve tronchuda, Brussels sprouts,
- *B. rapa:* turnip and turnip rapeseed,
- *B. napus:* rapeseed or colza and swedes, and
- *B. carinata*: Abyssinian mustard.

The project is divided in four subgroups covering the above-mentioned four *Brassica* species.

Annex 1 (page 54) lists the 16 partners from 8 European countries which participate in the project. Eleven partners are collection holders and conserve collections of the included *Brassica* species: two partners conduct mainly research into *Brassica* species and three partners are private plant breeding firms with programmes in some of the *Brassica* species, of which one partner includes 7 breeding companies.

The project is complementary to the activities coordinated within the framework of the ECP/GR Working Group on *Brassica*. So far three project meetings have been organized and the third (present) meeting has been jointly organized with the ECP/GR Working Group on *Brassica* from 8 to 9 February 2002 in Vila Real, Portugal.

Major activities of the project

The tasks and milestones of the project for the four years are clearly defined in the Technical Annex, part of the contract with the European Commission.

The objectives of the project can be summarized in five major activities:

A. Database and establishment of core collections of four Brassica species

- Updating the Bras-EDB with passport data of accessions not yet included
- Creation of core collections of the four *Brassica* species
- Linking the characterization and evaluation data to the database
- Ensuring access to this information.

B. Characterization and regeneration of the four core collections

- Characterization of the four *Brassica* species so as to complement the available data using minimum descriptors
- Characterization of *B. carinata* using DNA fingerprinting
- Regeneration of material of the four *Brassica* species with priority on the accessions included in the core collections
- Definition of minimum descriptors for *B. carinata*.

C. Evaluation of the accessions included in the core collections of the *Brassica* species

- Seeds from the core collection will be sent by the genebanks or institutes holding collections to the partners who carry out the evaluations
- Listed evaluations for disease and pest resistance, quality properties, salt tolerance and agronomic characters to be carried out
- Results of the evaluations to be linked to the Bras-EDB.

D. Rationalization, safety-duplication and recommendations for further collecting in Europe

- Passport data of the Bras-EDB to be made available for tracing duplicates
- Safety-duplication of the core collections and part of the other material will be completed
- Recommendations for collecting activities to be made.

E. Dissemination of information

- Publication of information by individual partners
- Dissemination of data through the Bras-EDB.

Results achieved after two years (mid-term assessment)

A. Database and establishment of core collections of four *Brassica* species

In order to make a complete new update of the Bras-EDB the project partners and other collection holders in Europe were requested to send new data sets to CGN. New data sets received from 29 institutions and the existing data from 3 collections, already included in the old version of the Bras-EDB, were the basis for the development of the new version. The new version of the Bras-EDB now includes 19 113 accessions. This means that data of 6113 new accessions have been added to the 1997 version (Boukema and Hintum 1998).

The EU partners of the GEN RES CT99 19-12 project hold 11 841 (62%) of the total number of 19 113 accessions presently included in the Bras-EDB. Tables with detailed information on the collections included in the Bras-EDB are presented in the paper of Boukema *et al.* on "The European *Brassica* Database: version 2001" (this volume, pp. 14-18.

The new version of the Bras-EDB can be found on CGN's homepage (www.plant.wageningen-ur.nl/cgn or www.genebank.nl).

The *Brassica* subgroups discussed the structure and size of the respective core collections during the first two project meetings. Two subgroups finalized the core collections and the *B. napus* group will finally reduce its core collection to approximately 150 accessions. The situation is presently as follows:

B. oleracea	396 accessions
B. rapa	100 accessions
B. napus	202 accessions (expected to become 150 accessions)

The subgroup *B. carinata* will establish the final core collection after the fingerprinting research and field characterizations have been finalized. Research to assist the development of the core collection was conducted in the second year. *Brassica carinata* (Abyssinian mustard) is not well known and not yet cultivated crop in Europe. Most of the accessions are populations collected in Ethiopia. This is the main reason why the development of the core collection differs from that of the other species.

Several partners of the project, particularly the genetic resources partners, provided seeds of the accessions from the core collections in order to conduct characterization and evaluation. Furthermore, partners also regenerated accessions included in the core collection to be used for characterization and evaluation in the next two years.

B. Characterization and regeneration of the four core collections

The four subgroups discussed the minimum descriptors at the second meeting in Córdoba (early 2001) and all subgroups agreed on the final descriptors.

Only parts of the collections have been characterized for minimal descriptors and major parts of the collections need regeneration. The collections are too extensive to regenerate, characterize and evaluate all accessions within the scope of this project. It is also believed that a large amount of duplication exists between collections. Therefore core collections of the four *Brassica* species have been developed, representing the diversity in the respective species. The accessions included in the cores will be given the highest priority for regeneration, characterization and evaluation.

Table 1 presents the characterizations finalized by the partners in 2001. It shows that in the second year 306 accessions were characterized. In total 10 of the 16 partners were involved in this activity. It should be noticed that the characterization of the initial core collection of *B. carinata* was conducted both in Spain and the United Kingdom.

Table 1. Overview of the characterizations completed by all subgroups in 2001

***Brassica* subgroup**	**No. of accessions characterized**	**No. of accessions regenerated**
B. oleracea	61 (+ 37 only partly)	45
B. rapa	53	53
B. napus	92	71
B. carinata	100 (twice at two locations)	32*
Total	**306 (+37 only partly)**	**201**

* plus 100 accessions selfed on 10 plants

Eleven partners of the project regenerated 201 accessions in 2001 (Table 1). Furthermore several partners started in 2001 with sowing and planting of biennial material for seed harvest in 2002.

C. Evaluation of the accessions included in the core collections of the *Brassica* species

The evaluation of germplasm is important for future utilization of genebank material in breeding programmes. Several private breeding firms are participating in these activities and have conducted trials in 2001. The evaluation concentrates on accessions included in the core collections of the four species. The project will conduct more than 1800 evaluations on different properties listed in Table 2.

So far, limited *Brassica* material has been evaluated for important properties such as resistance to various pests, diseases, environmental stress and quality properties such as glucosinolates and TAGs. Glucosinolates are sulphur compounds found in *Brassica* and other genera which after degradation can give rise to isothiocyanates. These isothiocyanates are active against microorganisms, insects, mites and nematodes. Plant residues from *Brassica* can therefore be used as a biofumigant. TAGs, particularly the pattern of the intact

triacylglycerol (TAG) and the fatty acid composition of the sn-2 position of the TAGs are very important for the improvement of seed oil quality.

Table 2 presents an overview of all evaluations conducted in 2001 and also indicates the evaluation activities which will be conducted in the next two years. It should be noted that the *B. napus* subgroup determined fatty composition of 1029 accessions, required to develop the core collection. This is much more than the size of the core of 150 accessions. As a result 2457 accessions have already been evaluated in the project for a large number of properties. These evaluations were carried out by 10 partners of the project. It is estimated that in the project more than 3000 evaluations will be conducted. It should also be noticed that evaluations on the *B. carinata* core collections have been conducted twice, in Spain and in the United Kingdom.

Table 2. Overview of the different evaluations conducted in 2001

Property concerned	*Brassica* species	Total no. of accessions planned	No. of accessions evaluated (2001)	Remarks
Pest /disease resistance				
Blackrot (*X. campestris)*	*B. oleracea*	Core (396 acc.)	396	Test in glasshouse
Mycosphaerella	*B. oleracea*	Core (396 acc.)	396	Natural infection
Clubroot (*P. brassicae)*	*B. oleracea*	Core (396 acc.)	198	Test in glasshouse
Clubroot(*P. brassicae)*	*B. napus*	Core (150 acc.)		Test in glasshouse
Field slugs	*B. napus*	Core (150 acc.)		Spring 2002
Stem weevils	*B. napus*	Core (150 acc.)		Sown autumn 2001
Downy mildew (*P. parasitica)*	*B. rapa*	Core (100 acc.)		Sown autumn 2001
White blister (*A. candida)*	*B. rapa*	Core (100 acc.)	100	*A. candida* isolate 7V
Flea beetles	*B. napus*	Core (150 acc.)		Sown autumn 2001
Flea beetles	*B. carinata*	Core (100 acc.)	100 (200)	Field damage
Quality_properties				
Fatty acid composition**	*B. napus*	Core (150 acc.)	1029*	Continued into 2003**
Glucosinolates (leaves)	*B. rapa*	Core (100 acc.)	36	8 different glucosinolates
Glucosinolates (leaves)	*B. napus*	Core (150 acc.)		Planned for 2002
Seed storage components	*B carinata*	Core (100 acc)	100 (200)	Seeds of two locations
Abiotic_characters				
Salt tolerance	*B. oleracea*	Core (100 acc.)	2	34 in trial 2001/2002
Agronomic characters				
Racemen, pod characters	*B. carinata*	Core (100 acc.)	100 (200)	Trials at two locations
Total			**2457**	

* Fatty acid composition on a large group of accessions was determined on material collected from a large field trial

** Pattern TAGs will be conducted on the core (150 accessions) in 2002-2003

D. Rationalization, safety-duplication and recommendations for further collecting in Europe

The new version of the Bras-EDB makes it easy to trace duplicates in the collections. On the basis of these findings, partners can set priorities for regeneration of their unique accessions.

During the project meeting in Córdoba the group again discussed the situation concerning safety-duplication. Not all partners have duplicated their material in other genebanks. Several partners offered storage facilities in their genebanks. Actions will be discussed again during the present meeting.

In its final year the project will formulate the need for further collecting in Europe. The Bras-EDB can be very helpful in making these recommendations.

E. Coordination and dissemination of information

The project organized three project meetings in Wageningen (The Netherlands), Córdoba (Spain) and Vila Real (Portugal). Some of the subgroups organized individual meetings.

Two progress reports were submitted to the European Commission and the publication of the third progress report over the second year is nearly finalized. Papers on the activities of the project will be published in the coming years by the individual partners. The papers included in this report are the first publicity given to the activities so far achieved by the project.

The new version of the Bras-EDB on the Internet has increased the access to *Brassica* collections in Europe.

Summary (mid-term assessment)

During the first two years important achievements of the project are as follows:

- A new version of the Bras-EDB was finalized and placed on the Internet. The Bras-EDB includes presently 19 113 accessions of 32 collections of 22 countries;
- the core collections of three *Brassica* species have been defined; the *B. carinata* subgroup is using an initial core collection and will define the final one later;
- minimal descriptors were agreed upon and the characterization was started as planned;
- all subgroups started to regenerate *Brassica* material;
- the project partners evaluated more than 2450 accessions for several properties;
- project members briefly discussed rationalization of the collection and some progress was made on the organization of safety-duplication; and
- the coordination of the project was carried out as planned without any real problems.

Acknowledgement

The work of this GEN RES CT99 109-112 project is supported by the European Commission in the framework of Council Regulation (EC) N° 1467/94.

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Annex I. Participants in the GEN RES CT99 109-112 project

P1	Centre for Genetic Resources, The Netherlands, Wageningen, The Netherlands
P2	Genetic Resources Unit, Horticulture Research International, Wellesbourne, UK
P3	Nordic Gene Bank, Alnarp, Sweden
P4	Institut National de la Recherche Agronomique, Rennes, France
P5	Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany
P6	National Agricultural Research Foundation, Agricultural Research Center of Makedonia and Thraki, Greek Gene Bank, Thessaloniki, Greece
P7	Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (DOFATA), Università degli Studi di Catania, Italy
P8	Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal
P9	Consejo Superior de Investigaciones Cientificas, Mision Biologica de Galicia, Pontevedra, Spain
P10	Federal Centre for Breeding Research on Cultivated Plants, Gene Bank, Braunschweig, Germany
P11	Norddeutsche Pflanzenzucht Hans Georg Lembke KG, Hohenlieth, Holtsee, Germany
P12	Deutsche Saatveredelung Lippstadt - Bremen Gmbh, Lippstadt, Germany
P13	Universidad Politechnica Valencia, Valencia, Spain
P14	Plantum NL, representing 7 breeding firms, Gouda, The Netherlands.
P15	Consejo Superior de Investigaciones Cientificas, Instituto de Agricultura Sostenible, Córdoba, Spain
P16	John Innes Centre, Norwich, UK

Role and progress of the *Brassica oleracea* group in the GEN RES CT99 109-112 project after 2 years

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Introduction

In the GEN RES CT99 109-112 project "*Brassica* collections for broadening agricultural use, including characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop", four subgroups were formed (van Soest *et al.*, this volume, page 50). The *B. oleracea* subgroup includes the following partners:

- Centre for Genetic Resources, Wageningen, the Netherlands (*sub-coordinator)* (CGN)
- Institut National de la Recherche Agronomique, Rennes, France (INRA)
- National Agricultural Research Foundation, Agricultural Research Centre of Macedonia and Thraki, Greek Gene Bank, Thessaloniki, Greece (GGB)
- Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, Università degli Studi di Catania, Italy (DOFATA)
- Universidad Politechnica Valencia, Valencia, Spain (UPV)
- Plantum NL, Gouda, The Netherlands (Plantum)

B. oleracea is a very diverse species and includes different types (see Table 1). The aims of the project are presented in the above-mentioned paper of van Soest *et al*.

Role of the B. oleracea *subgroup in the project*

The role of the *B. oleracea* subgroup can be summarized as follows:

- update the Bras-EDB with passport data
- develop a *B. oleracea* core collection
- characterize part of the collections according to agreed minimum descriptors
- define and regenerate material, giving priority to the accessions in the core collection
- evaluate (part of) the core collection for resistance to *Plasmodiophora brassicae, Xanthomonas campestris* and *Mycosphaerella brassicicola* and for salt tolerance.

Results achieved after two years

Updating the Bras-EDB with passport data

All genetic resources partners of the *oleracea* subgroup have sent their passport data for inclusion in the new version of the Bras-EDB. In the new version of the Bras-EDB, *B. oleracea* is by far the largest group with over 10 000 accessions (Boukema *et al.*, this volume, page 16). This species represents 54% of the total number of accessions.

B. oleracea core collection

In the start-up meeting it was decided that the existing *B. oleracea* core collection (Boukema *et al.* 1997), developed in a former EU *Brassica* project, would be used as a starting point for the creation of the new core collection. The numbers per crop group, per country and per type were discussed and adjusted. All partners holding *B. oleracea* collections identified material from their collections, representing a broad variation of material from their country or region and fulfilling the needs of the evaluation partners regarding quality and quantity of the seeds. Wild *Brassica* species (n=9) related to *B. oleracea* were donated by Dr Cesar Gomez-Campo from Universidad Politecnica de Madrid (UPM). The new core collection includes

396 accessions. An overview is given in Table 1. Seeds of all accessions included in the core collection were distributed to the evaluation partners.

Table 1. *B. oleracea* core collection per donating genebank (see for explanation van Soest *et al.,* this volume, pp. xx) and *B. oleracea* group

Common name *B. oleracea* group	No. of acc.	P1 CGN	P2 HRI	P3 NGB	P4 INRA	P5 IPK	P6 GGB	P7 IDG	P9 MBG	P10 BGRC	P13 UPV	UPM
Kale	79	18	26	3	10	7		1	10	2	2	
Chinese kale	10	5	5									
Cauliflower	63	18	21	2	11	2		8			1	
Broccoli	35		22			2		8			3	
Cabbage	100	33	30	5	5	16	2		4		5	
Brussels sprouts	40	13	22	4		1						
Kohlrabi	20	4	7			7		2				
Tronchuda	15	2	12						1			
Unknown	20	5									15	
Wild *oleracea*	3	1										2
Wild related species n=9	11	1				2						8
Total	**396**	**100**	**145**	**14**	**26**	**37**	**2**	**19**	**15**	**2**	**26**	**10**

Characterization and regeneration

In the start-up and second meeting the minimum descriptors used in this project were discussed. The basis was the "Descriptors for *Brassica* and *Raphanus*" (IBPGR 1990) and the report of the second meeting of the Working Group on *Brassica* in 1994, Lisbon, Portugal (Gass *et al.* 1995). Some traits were adapted and some added and final conclusions were made at the second year's progress meeting (Table 2). A definitive list with the descriptors used in all *Brassica* groups was distributed to all partners. It was decided that a minimum of 30 plants would be used for characterization.

All partners produced overviews including material from their respective collections which required regeneration. Priority was given to material included in the core collection.

In total 61 accessions were described. In addition 37 accessions were partly characterized. A total of 45 accessions were successfully regenerated. In 2001 material was sown for characterization and regeneration in 2002.

Evaluation

Methods for the evaluation of the *B. oleracea* core collection were discussed at the start-up meeting and the protocols defined. So far 198 accessions of the core collection have been screened for resistance to *Plasmodiophora brassicae* (clubroot). The screening was successful but only a few accessions show high levels of resistance. 396 accessions were screened for *Xanthomonas campestris* pv. *campestris* (blackrot) as well as for *Mycosphaerella brassicicola* resistance. Preliminary investigations show that in several accessions of different *Brassica oleracea* types, plants resistant to *Xanthomonas* were found. However, the percentage of resistant plants in these accessions was in general very low. The highest percentage of resistant plants was found in a cauliflower accession (71-75% resistant plants). Only a few genotypes with 100% resistant plants to *Mycosphaerella* were found in the field, particularly in red cabbage.

For salt tolerance the evaluation of two accessions was completed and results of the 32 other accessions will follow early in 2002.

Table 2. Minimum descriptors for *B. oleracea*, based on the list *in* Gass *et al.* (1995)

Crop type	Descriptor	Descriptor no.*	Remark**
Cabbage	Leaf blade blistering/crimping	4.2.21	
	Leaf colour	4.2.24	
	Head shape	4.2.35	
	Head length/diameter ratio	4.2.43	
	Stem length under head	4.2.57	
Kales	Plant growth habit	4.2.2	
	Leaf colour	4.2.24	
	Stem length/diameter ratio	4.2.56	
	Leaf blade curling	4.5.10**	New; 0=none, 3=weak, 5=medium, 7=strong
Tronchuda	Leaf colour	4.2.24	
	Petiole and/or midvein enlargement	4.2.27	
	Head shape	4.2.35	
	Head-forming leaf overlap at terminal region	4.2.36	
	Stem length under head	4.2.57	
Cauliflower	Leaf colour	4.2.24	
	Head cover from subtending leaves	4.2.37	
	Curd shape (flowering head shape in longitudinal section)	4.2.75	
	Curd colour	4.2.78	
	Harvest time or season	4.5.12**	New; 0=no cold requirement, 1=low, 2=high cold requirement
	Time from planting to harvest	4.5.13**	New; 1=early (<60 days) ; 2=medium (60-120 days) ; 3=late (>120 days)
	Flowering head solidity	4.2.77	added
Broccoli	Leaf colour	4.2.24	
	Floral apex branching pattern	4.2.73	
	Flowering head solidity	4.2.77	
	Flowering head colour surface	4.2.78	
	Plant growth habit	4.2.2, states 7 and 8	added
Brussels sprouts	Leaf colour	4.2.24	
	Sprout distance (number of buds per unit of stem)	4.2.64	
	Firmness of enlarged vegetative buds	4.2.67	
	Sprout colour	4.2.68	
Kohlrabi	Stem length/diameter ratio	4.2.56	
	Stem colour	4.2.60	
	Leaf retention on stem	4.2.61	
	Stem, shape in longitudinal section	4.5.4**, use states of 4.2.82	
All crops	Overall uniformity	4.5.14**	New; 1=completely uniform; 2= not uniform; 3=every plant different
	Additional site data	3.1 – 3.7	

* Descriptor number from "Descriptors for *Brassica* and *Raphanus*" (IBPGR 1990)

** New descriptors by Mats Gustafsson and Gert Poulsen, but descriptor states changed during the project meetings

Summary (mid-term assessment)

The milestones defined for the first two years have been fulfilled. Important achievements of the *Brassica oleracea* subgroup in the project are:

- the core collection of *Brassica oleracea* has been established;
- minimal descriptors were agreed upon and the characterization and regeneration was started as planned;
- the core collection has been successfully evaluated for resistance to two diseases and will be for a third by the end of the project. Part of the core collected has been tested for salt tolerance.

Acknowledgement

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IBPGR. 1990. Descriptors for *Brassica* and *Raphanus*. International Board for Plant Genetic Resources, Rome. 51pp.

Progress of the *Brassica rapa* group in the GEN RES CT99 109-112 project

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The work programme of the EU project "*Brassica* collections for broadening agricultural use" is based on four crop-specific groups. The role and activities of the *Brassica rapa* group are described below at the mid-point after 2 years.

The Brassica rapa *group*

There are 4 project partners involved in the *Brassica rapa* subgroup, namely:

- Horticulture Research International, UK (Angela Pinnegar/Dave Astley)
- Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany (Klaus Dehmer)
- Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal (Eduardo Rosa)
- Mision Biologica de Galicia, Pontevedra, Spain (Armando Ordas/Elena Cartea).

The tasks of the *B. rapa* subgroup relate to various work areas including:

- documentation and updating the Bras-EDB
- establishment of a project *B. rapa* core collection and making it available for use
- characterization of the *B. rapa* core collection for the agreed project minimum descriptors
- regeneration of the *B. rapa* core collection
- an initial evaluation of the core collection for pathology and glucosinolates
- safety-duplication (begun, with material sent to the appointed centres)
- technical and financial reporting to the European Commission.

The achievements of the *B. rapa* subgroup within the project are summarized below.

Documentation

Passport data from all subgroup partners were donated to the central European database (Bras-EDB) thus fulfilling the objective.

Brassica core collections

Brassica rapa

The project *B. rapa* core collection of 100 accessions has been established and the taxonomic identity of the representatives verified using cytometry. Two accessions in the original core "pool" were discarded following this validation procedure. A third accession was identified as a tetraploid *B. rapa*, and this accession has been retained in the core collection. When sufficient seeds were available the accessions of the core collection were tested for germination. The *B. rapa* accessions that had only few seeds available were scheduled for regeneration by the subgroup partners. The *B. rapa* core collection has been used to initiate the evaluation work in the project. Table 1 shows the breakdown of numbers of accessions according to their taxonomy and donor institute.

Table 1. GEN RES *Brassica rapa* core collection - March 2002

Taxonomic breakdown	No. of accessions
Brassica rapa (undefined)	9
Brassica rapa chinensis	5
Brassica rapa chinensis chinensis	5
Brassica rapa chinensis rosularis	5
Brassica rapa dichotoma	4
Brassica rapa nipposinica	2
Brassica rapa nipposinica chinoleifera	3
Brassica rapa oleifera	7
Brassica rapa oleifera annua	4
Brassica rapa oleifera biennis	6
Brassica rapa pekinensis	11
Brassica rapa pekinensis glabra	5
Brassica rapa pekinensis laxa	4
Brassica rapa rapa	20
Brassica rapa ruvo	7
Brassica rapa trilocularis	3
Total	**100**

Donor Institutes*	
CHERAC	1
DEUBGRC	6
DEUIPK	22
DEUIPKM	7
ESPMBG	3
HRIGRU	22
NLDCGN	18
PRTEAN-ESPCRF	1
REGNGB	6
SUNVIR	6
TURAARI	2
USDA via DEUBGRC	6
Total	**100**

***Acronyms**

CHERAC	Station Fédérale de Recherches Agronomique de Changins, Nyon, Switzerland
DEUBGRC	Federal Centre for Breeding Research on Cultivated Plants (BAZ) – Gene Bank, Braunschweig, Germany
DEUIPK	Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany
DEUIPKM	Institut für Pflanzengenetik und Kulturpflanzenforschung, Malchow, Germany
ESPCRF	Centro de Recursos Fitogeneticos, Instituto Nacional de Investigación y Technologia Agraria y Alimentaria (CRF-INIA), Madrid, Spain
ESPMBG	Mision Biologica de Galicia, Pontevedra, Spain
HRIGRU	Genetic Resources Unit, Horticulture Research International, United Kingdom
NLDCGN	Centre for Genetic Resources the Netherlands
PRTEAN	Estação Agronomica Nacional, Oeiras, Portugal
REGNGB	Nordic Genebank, Alnarp, Sweden
SUNVIR (=RUSVIR)	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russia
TURAARI	Aegean Agricultural Research Institute, Izmir, Turkey
USDA	United States Department of Agriculture, USA

Brassica oleracea and *Brassica napus*

In consultation with the subcoordinators for the *Brassica oleracea* (Ietje Boukema) and *Brassica napus* (Gert Poulsen) core collections, the *B. rapa* subgroup partners provided seeds and associated information for use in the respective evaluation programmes.

Characterization of the *B. rapa* core collection for the agreed minimum descriptors

The draft list of descriptors developed by the subgroup partners in year 1 of the project was considered further at the annual project meeting in Córdoba. The attached Table 2 lists the minimum descriptors agreed by the *B. rapa* subgroup for use in the project characterization.

Three of the subgroup partners were involved in characterization using the minimum descriptors. In the current year (2001), a total of 53 accessions of *B. rapa* have been characterized accounting for >50% of the *B. rapa* core collection. This fulfils the characterization milestone for the *B. rapa* subgroup in the project thus far.

Table 2. *B. rapa* minimum characterization descriptors (draft). Based on the "Descriptors for *Brassica* and *Raphanus*" (IBPGR 1990), the published minimum descriptors for *B. rapa* of the ECP/GR *Brassica* Working Group (Gass *et al.* 1995) and UPOV descriptors (TG/37/7) (UPOV 1988) as appropriate.

Descriptor	Code
Site and trial	
Country	3.1
Site (Research Institute)	3.2
Name of person in charge	3.3
Sowing date	3.4
Transplant date	3.5
First harvest date	3.6
Last harvest date	3.7
General	
Ploidy (optional)	UPOV (2.)
Turnip root form	
Organ used as primary product	2.20
Major crop usage	2.22
Root shape	4.2.82
Root colour of skin above ground	4.2.92.1
Root colour of skin below ground	4.2.92.2
Interior root colour (flesh)	4.2.93
Leaf lobes	UPOV (9.)
Turnip tops/greens – leaf forms	
Plant growth habit	4.2.2
Leaf colour	4.2.24
Petal colour	4.3.17
Leaf lobes	UPOV (9.)
Flowering plant, degree of branching (to be assessed at first open flower)	4.3.13
Turnip oilseed rape	
Leaf colour	4.2.24
Stem colour	4.2.60
Petal colour	4.3.17
Time of flowering (spring sown)	UPOV (39.)
Tendency to form inflorescence (spring sown)	UPOV (38.)
or	
Flowering behaviour under normal crop conditions	4.3.1
Asian leaf/head types	
Plant growth habit	4.2.2
Leaf colour	4.2.24
Leaf hairiness	4.2.25
Petiole and/or mid-vein enlargement	4.2.27
Head-forming leaf overlap at terminal region	4.2.36

Regeneration of the *B. rapa* core collection

Three of the subgroup partners (HRIGRU, IPK and MBG) and the Centre for Genetic Resources, the Netherlands (CGN-P1) were involved in the regeneration of *B. rapa* in 2001. The inputs into regeneration for 2001 can be divided on the basis of the life cycle characteristics of the *B. rapa* material, i.e. annual or biennial. For annual *B. rapa* 53 accessions have been grown, and seed produced. A further 40 accessions of biennial *B. rapa* have been sown and transplanted, enabling the plants to be vernalized over winter for seed production in 2002. The expected output defined in the project Technical Annex is 55. The shortfall of 2 accessions was caused by one seed batch not germinating and the other accession turning

out to be biennial, for which seed will be produced in 2002. Seed was produced for 6 annual accessions in 2000, therefore overall the *B. rapa* regeneration is more or less on target.

Initial screening of the core collection

There are 2 partners involved in the evaluation of *B. rapa* in the project, namely HRI for pathology screening and the Universidade de Trás-os-Montes e Alto Douro, Vila Real for biochemical screening.

- **Pathology:** the *B. rapa* core collection of 100 accessions was screened for reaction to the *Albugo candida* isolate 7V by Eddie Byrne and Pam Gordon at HRI Wellesbourne. The results show that there is a wide range of responses in the *Brassica rapa* accessions ranging from 100% susceptibility to complete resistance. Interestingly, 20% of the accessions showed a resistant response with more than 80% of individuals with no pustules. However this final category may not reflect the true resistance of the accessions screened because in some cases the control plants within each seed tray scored less than 50% sporulation. In each case where sporulation on the control was low, the zoospores were reported to have not become fully motile. Despite these inconsistencies, the results are still extremely encouraging.
- **Glucosinolates:** 36 accessions of the *B. rapa* core collection were grown under field and controlled-environment conditions. Sub-samples of tissue were taken from all plants for each accession. The tissue samples for each accession were homogenized in liquid nitrogen and freeze-dried for analysis. The material was screened quantitatively for the presence of 8 glucosinolates, namely 2-Hydroxybut-3-enyl; 2-Propenyl; But-3-enyl; Pent-4-enyl; Indol-3-ylmethyl; 2-phenethyl; 4-Methoxyindol; 1-Methoxyindol-3-ylmethyl. The results were presented in tabular form in the project report to the EU.

Safety-duplication

The system for the safety-duplication of the *B. rapa* collections was agreed by the subgroup during the project start-up meeting in CGN, Wageningen. The partners involved in regeneration have already developed black box bilateral agreements (formal and informal) for their safety-duplicates with other partners or genebanks with suitable agreed standard storage conditions.

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Development and evaluation of a *Brassica napus* L. core collection

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The major objectives of the *Brassica napus* subgroup in the GEN RES CT99 109-112 project are to:

- Update the European *Brassica* Database (Bras-EDB) and establish a *Brassica napus* L. core collection of 150 accessions
- Characterize the core collection by morphological descriptors
- Evaluate the core collection for agronomically important traits (Lühs *et al.*, this volume, pp. 67-71).

Updating the Bras-EDB and establishment of four core collections

The updating of the database was easily accomplished and very urgent for the group as the large IPK collection at Malchow Branch Station was not previously included. Additionally, we found that important information was missing in the Bras-EDB, which led us to perform two field screenings of all available European *Brassica napus* material; this will be discussed later.

Establishment of the Brassica napus *core collection*

At the start of the project the Bras-EDB contained 2240 *B. napus* accessions. After adding the 643 accessions from IPK-Malchow it held 2883 accessions. It was cleared for name duplicates, breeding lines, other taxa and material without sufficient information, ending up with 953 accessions. Eight Chinese accessions are also tested.

A preliminary core collection of 188 accessions (Table 1) was established and distributed for characterization. Eventually, after the characterizations and evaluations have been carried out, the accessions will be selected to leave a collection of 150 accessions.

Table 1. Division of *Brassica napus* accessions into subgroups according to end use. The Bras-EDB composition and the corresponding accessions in the preliminary core collection

END_USE	No. of accessions	Core collection composition
Oilseed rape, spring-type	151	25
Oilseed rape, winter-type	394	75
Forage rape, spring-type	41	6
Forage rape, winter-type	30	20
Swede	258	35
Vegetables	51	12
Others	28	15
Total	**953**	**188**

Within each of the END_USE groups the core collection entries were intended to be represented proportionally to the number of accessions from each country. Within these country groups the material was selected so that German varieties were selected preferentially from the German genebanks, hoping for higher authenticity this way. Accessions with local names were selected anticipating local origin and thus securing high diversity in the core collection. Likewise, for the large groups of oilseeds, early materials from different breeders were selected and in addition some contemporary varieties with a low content of erucic acid and glucosinolates were included.

Characterization of the core collection

The characterizations started with the field trial carried out in Rauischholzhausen/Hesse, Germany in summer 2000. All available *B. napus* material from the Bras-EDB was grown to give an impression of the diversity in the material, compare name duplicates and supplement database data with information on vernalization requirements (Table 2), flowering time, etc. In 2000 the field trial comprised 338 putative summer types of *B. napus*, and the trial for winter type material was carried out in 2001, including 857 accessions.

Table 2. Descriptors to differentiate among *B. napus* forms for flowering ability and winter hardiness

Winter hardiness	Form	Seasonal type	Use	Descriptor
Hardy	*perennis*			5
	biennis	winter biennial	oil	4
	biennis	winter annual	forage	3
Not hardy	*biennis*	summer annual	forage	2
	annua	summer annual	oil/forage	1

The next step was to define the minimum descriptors to be used for the characterization of the three main groups of the *B. napus* core collection i.e. the oilseeds (Table 3), the swedes (Table 4) and the vegetable types.

The minimum descriptor lists are selected from previously defined descriptors, published by IBPGR (1990) with subsequent revisions and UPOV (1996) except for the "vernalization requirement and seasonality" descriptor (Table 3). The descriptors for the leafy vegetable types of *B. napus* were adopted from the *B. oleracea* subgroup kale descriptors.

Table 3. Minimum descriptors selected for characterization of *B. napus* oilseed and forage rape

Character	Source
Anthocyanin coloration	
- flower stem	IBPGR 4.2.60
- petiole and midvein	IBPGR 4.2.33
Leaf, number of lobes	UPOV 6
Leaf colour	IBPGR 4.2.24, UPOV 4,
Flowering time	UPOV 11
Petal colour	IBPGR 4.3.17, UPOV 12
Vernalization requirements and seasonality	IPGRI 4.5.16

Table 4. Minimum morphological descriptors selected for characterization of swedes

Character	Source
Root shape	IBPGR 4.2.82
Root colour of skin above ground	IBPGR 4.2.92.1
Root colour of skin below ground	IBPGR 4.2.92.2
Interior root colour	IBPGR 4.2.93
Leaf, number of lobes (optional)	UPOV 6

This season (2001), 13 accessions of spring oilseed rape, 37 accessions of winter oilseed rape (Fig. 1) and 42 swede accessions were characterized. The swedes were characterized using the descriptors listed in Table 3 and analyzed using multivariate analyses creating the similarity dendrogram presented in Fig. 2.

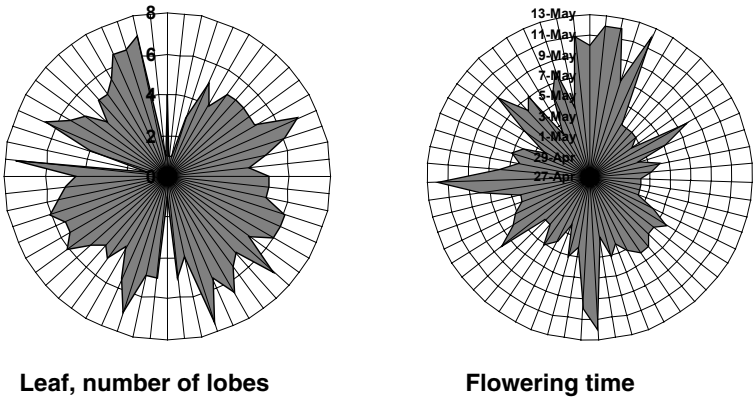

Fig. 1. Diversity of winter oilseed rape accessions characterized by BAZ.

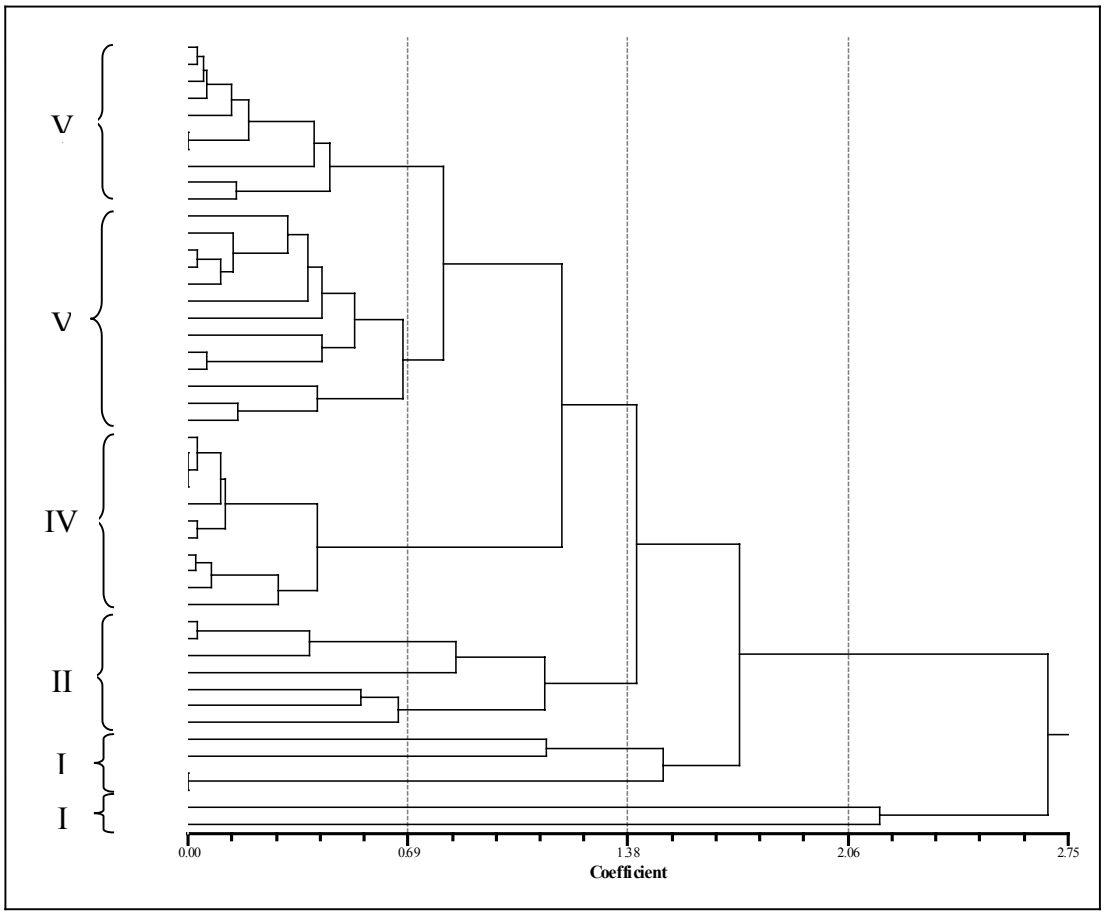

Fig. 2. Diversity among swede accessions in the *Brassica napus* core collection characterized by 5 morphological descriptors and subjected to UPGMA-cluster analysis using the NTSYS package.

The analysis demonstrates the diversity among the selected swede accessions. Six main clusters are identified. The first cluster is characterized by white internal root colour, and cluster 2 accessions have unlobed leaves or only few lobes. Accessions in cluster 4 have reddish skin colour below the ground, while cluster 6 is distinguished by green skin colour above the ground. Cluster 5 is distinguished from cluster 6 in skin colour above ground and from cluster 4 on the yellow skin colour below the ground. Cluster 3 is less clear; it varies from cluster 4 in that the root colour of below-ground skin is predominantly white and from cluster 2 in having more lobes on the leaves.

This kind of analyses will be applied to all groups of the core collection to evaluate its success in reflecting the diversity of the crop.

The core collection is simultaneously being evaluated for a number of traits of agronomic relevance. The status of this part of the project is described in "Preliminary field evaluation of a *Brassica napus* core collection" (Lühs *et al.*, this volume, pp. 67-71).

The extensive phenotypic and quality data created in this project will be used in selecting accessions representing the diversity within the species *B. napus* and to establish a European core collection of 150 accessions.

The core collection is not a static unit and it is our hope that in the future it will be possible to continue the development of the collection by adding more evaluation data of new traits and including material to fill out gaps which will be discovered during this work.

Acknowledgements

In selection of the swede entries, discussions with Niall Green, Scottish Agricultural Science Agency, have improved the composition of the collection and facilitated the work. The authors gratefully acknowledge financial support for their work, which is part of the EU-funded project GEN RES CT99 109-112 "*Brassica* collections for broadening agricultural use, including characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop".

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Preliminary field evaluation of a *Brassica napus* core collection

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Brassica crop species have become one of the most important sources of oil, condiments, vegetables, forage and green manure worldwide (Lühs *et al.* 2002). To optimize the exploitation of genetic resources of *Brassica* crops in plant breeding, the EU-funded project GEN RES CT99 109-112 was initiated. This joint project aims at conserving, documenting, characterizing, evaluating and rationalizing European collections of the crop species *Brassica napus* L., which encompasses oilseed rape varieties and some fodder crops (subsp. *napus*), rutabaga or swede turnips (subsp. *napobrassica* (L.) Hanelt) as well as "exotic" types, including vegetables (subsp. *pabularia*), Hakuran, and some Couve Nabica types. As a first step, a preliminary *B. napus* core collection had to be created representing the broad variation found among the accessions in the Bras-EDB (cf. Poulsen *et al.*, this volume, pp. 63-66). Besides morphological and quality assessment of the material, the main task is the evaluation of the core collection resistance to clubroot disease (*Plasmodiophora brassicae*) and pests, such as flea beetles (*Psylliodes chrysocephela, Phyllotreta* spp.), stem weevils (*Ceutorhynchus* spp.) and field slugs (*Deroceras* spp.).

Gross evaluation of B. napus *accessions*

Basic differentiation of the material

With the objective of creating a *B. napus* core collection, a great deal of information about the growth habit, use and seed quality of the material recorded in the database had to be collected. For a basic differentiation of the *B. napus* accessions (excluding subsp. *napobrassica*), the characters winter hardiness, vernalization requirement, seasonality and type of use were used (cf. Poulsen *et al.*, ibid).

Following this gross grouping of the material, 338 summer type *B. napus* accessions in 2000 (Table 1) and in the 2000-2001 growing season a total of 857 *B. napus* winter type accessions (Table 2) were grown at the Field Research Station in Rauischholzhausen near Marburg/Hesse, Germany. The later trial included genotypes displaying a biennial growth habit but remaining vegetative like forage types in the preceding spring trial.

Table 1. Spring field trial involving *Brassica* genebank material (2000)

Field No.	Use
1-198	Putative oilseed rape types
199-296	Putative fodder and green manure types
297-338	"Exotic" types, incl. vegetables (subsp. *pabularia*), Hakuran, Couve Nabica

Check, oilseed rape: cv. 'Star'
Check, fodder rape: cv. 'Jumbo' (f. *annua*), cv. 'Caramba' (f. *biennis*)

Due to a relatively mild winter in 2000-2001, nearly all of these accessions survived and showed transition to the generative stage. In the winter trial the oilseed rape cultivars 'Ceres', 'Falcon' (both double-low quality) and 'Maplus' (high erucic, low glucosinolate) were used as standards for oil types and cvs. 'Akela' and 'Caramba' as standards for fodder types (Table 2). In addition to basic description of this large set of *B. napus* material (excluding subsp. *napobrassica*), plant growth morphology and generative characters were evaluated, viz. beginning/end of flowering and harvest time.

Table 2. Winter field trial involving *Brassica* genebank material, 2000-2001

Field No.	Use
1-480	Putative oilseed rape types
481-707	Putative fodder and green manure types,
708-807	Fodder types, exotic types (staying vegetative, spring trial)
808-857	Not specified

Check, oilseed rape: cv. 'Ceres', 'Falcon' and 'Maplus'
Check, fodder rape: cv. 'Akela' (f. *biennis*) and cv. 'Caramba' (f. *biennis*)

Seed quality analyses

For seed quality analyses in each plot three to five plants were isolated with bags to obtain seeds for fatty acid analysis by gas chromatography (GLC), while the open-pollinated seed material was collected for oil, protein and glucosinolate (GSL) analysis by near-infrared reflectance spectroscopy (NIRS). Table 3 shows the results of seed quality analyses obtained for the seed material harvested from the spring field trial.

Table 3. Gross composition of harvested seed material derived from the spring field trial (2000) involving *Brassica* genebank material (N = number of samples; OP = open-pollinated, SF = selfed seed)

Constituent	N	OP/SF	Min.	Mean	Max.	CV%
Oil (% ADM, NIRS)	202	OP	35.1	40.8	48.3	5.7
Protein (% ADM, NIRS)	202	OP	23.4	26.7	30.9	6.0
GSL (µmol/g seed, NIRS)	202	OP	7.5	39.5	84.9	59.2
Palmitic acid (%, GLC)	332	SF	2.1	4.4	8.7	25.6
Stearic acid (%, GLC)	332	SF	0.1	2.2	4.5	32.3
Oleic acid (%, GLC)	332	SF	10.1	48.3	74.9	39.0
Linoleic acid (%, GLC)	332	SF	8.9	19.6	32.8	26.8
Linolenic acid (%, GLC)	332	SF	3.4	7.9	17.4	29.2
Eicosenoic acid (%, GLC)	332	SF	0.1	4.4	19.6	118.9
Erucic acid (%, GLC)	332	SF	0.1	12.5	58.1	148.1

ADM = absolute dry matter basis; NIRS = near-infrared reflectance spectroscopy; GLC = gas chromatography.

When breeding *Brassica* crops, the erucic acid content in the seed oil and the glucosinolate content of the meal are very important quality characteristics. The genebank accessions could be classified as follows: 1) high erucic acid-high glucosinolate (HEAR); 2) low erucic acid-high glucosinolate (LEAR); 3) high erucic acid-low glucosinolate; and 4) low erucic acid-low glucosinolate (double low quality, canola). The erucic acid content, which was

determined in selfed seed material from individual plants of accessions included in the spring trial, showed considerable variation. Following the differentiation of the material into HEAR and LEAR/canola types, in both major quality groups off-types (low-erucic types in the HEAR group and high erucic individuals in the LEAR/canola group) were observed. A similar variation was found for the low-glucosinolate spring rape cultivar 'Bronowski', which is known to have an erucic acid content of 8-10% and displayed a range of 0-35% (Fig. 1). The seed analyses show that the seed material obtained from different European genebanks may have been contaminated during seed regeneration due to cross-pollination (Lühs *et al.* 2003).

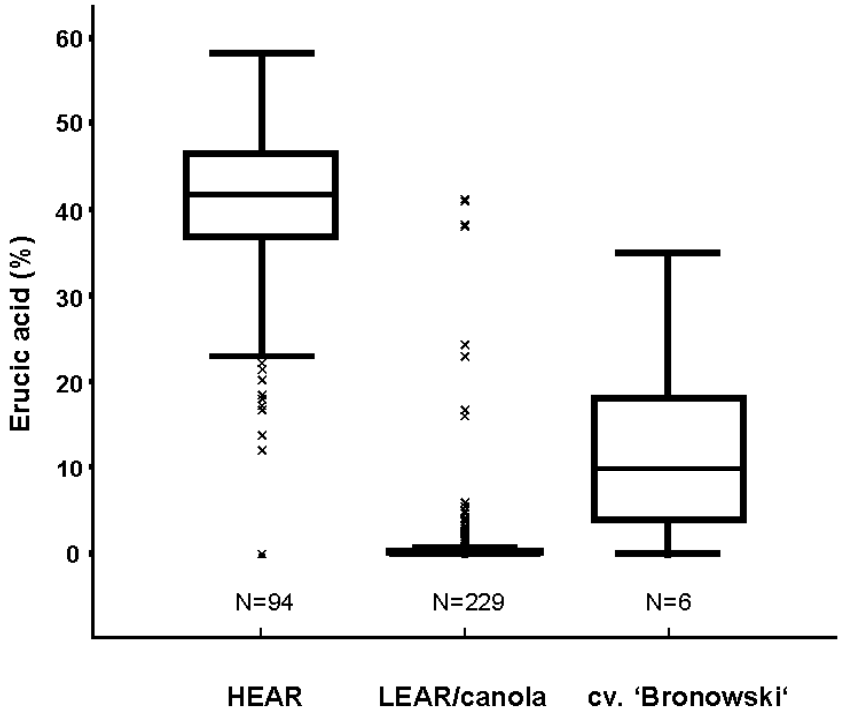

Fig. 1. Variation of erucic acid content in selfed seed material from individual plants of *B. napus* accessions (N = number of individual plants), grouped according to their seed oil quality in HEAR and LEAR/canola types as well as different accessions of cultivar 'Bronowski' (Lühs *et al.* 2003).

Table 4 summarizes the results of NIRS seed quality analyses obtained for the seed material harvested from the winter field trial (2000-2001). Regarding oil, protein and glucosinolate content the genebank material displays considerable variation as compared to the check varieties. In general, the material shows the well-known inverse relationship of oil and protein content with the consequence that the sum of protein and oil is nearly stable. As demonstrated by the checks representing current cultivars, rapeseed breeders have directed their quality selection towards oil content improvement and reduction of glucosinolates (Table 4).

Seed quality data and extensive phenotypic results of both field trials were used to select accessions for a preliminary core collection of about 180 specimens (incl. subsp. *napobrassica*) representing the variability within the species *B. napus* (Poulsen *et al.*, ibid.).

Table 4. Gross composition of harvested seed material derived from the winter field trial (2000-2001) involving *Brassica* genebank material and check varieties (N = number of samples; OP = open-pollinated, SF = selfed seed)

Constituent	N	OP/SF	Min.	Mean	Max.	CV (%)
Genebank material						
Oil (% ADM, NIRS)	2534	SF	30.3	46.3	57.2	9.7
Oil (% ADM, NIRS)	827	OP	33.5	48.1	57.8	7.7
Protein (% ADM, NIRS)	2534	SF	17.5	24.6	34.7	11.9
Protein (% ADM, NIRS)	827	OP	14.5	22.6	31.9	12.2
GSL (µmol/g seed, NIRS)	2534	SF	2.0	64.5	125.0	43.8
GSL (µmol/g seed, NIRS)	827	OP	0.8	68.0	123.5	42.3
Check varieties						
Oil (% ADM, NIRS)	28	SF	41.2	49.0	55.1	7.3
Oil (% ADM, NIRS)	17	OP	48.9	52.4	57.0	5.3
Protein (% ADM, NIRS)	28	SF	17.4	22.5	27.3	12.5
Protein (% ADM, NIRS)	17	OP	16.7	19.1	23.9	8.8
GSL (µmol/g seed, NIRS)	28	SF	7.2	13.6	54.6	62.4
GSL (µmol/g seed, NIRS)	17	OP	4.7	8.4	14.1	34.3

ADM = absolute dry matter basis; NIRS = near-infrared reflectance spectroscopy

Evaluation with respect to phytopathological traits

Depending on the biology of the respective pests, winter oilseed rape is attacked twice by insects: in the seedling stage by cabbage stem flea beetle (*Psylliodes chrysocephela*) and in early spring by larvae of stem weevils (*Ceutorhynchus napi*, *C. quadridens*) mining the stems of the plants. *Phyllotreta* flea beetles on the other hand, are insect pests of exclusively spring varieties (Lamb 1989). As breeding of double-low cultivars of oilseed rape has successfully reduced the seed glucosinolate content, some secondary plant compounds have gained importance with regard to certain pests. In the green matter glucosinolates are beneficial due to their function as feeding deterrents or toxins for polyphagous herbivores, such as field slugs (*Deroceras* spp.). On the other hand, glucosinolates or their fission products are involved as attractants in interactions with specialized insects feeding and/or reproducing on cruciferous crops (Glen *et al.* 1990; Giamoustaris and Mithen 1995). Therefore, it could be of interest to analyze the material for glucosinolate composition when considerable variation exists in the response to the above-mentioned pests. In the present study the rapeseed plants, sown in randomized field plots in 2001-2002, will be screened for flea beetle damage, which is measured by both feeding damage (percentage of leaf area missing) of adult insects in the autumn and the counting of larvae in the stem/leaf petioles. Damage by adult stem weevils (*Ceutorhynchus* spp.) is caused by egg laying in early spring (percentage of stems with boreholes) and the occurrence of larvae/eggs in the stem. Regarding the damage by field slugs (*Deroceras* spp.), in the autumn the slugs have to be counted and the percentage of plants attacked and the missing leaf area are determined.

Summary and outlook

The extensive phenotypic and quality data have been used to select accessions representing the variability within the species *B. napus* and to establish a reliable European core collection of 150-200 accessions including swede turnip or rutabaga types (subsp. *napobrassica*). During the 2001-2002 growing season the *B. napus* subgroup of the GEN RES project evaluated the core collection for resistance to clubroot disease (*Plasmodiophora brassicae*) and important pests including flea beetles (*Psylliodes chrysocephela*) and stem weevils (*Ceutorhynchus* spp.).

Acknowledgements

The authors gratefully acknowledge financial support for their work, which is part of the EU-funded project "*Brassica* collections for broadening agricultural use, including

characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop" (GEN RES CT99 109-112).

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Role and progress of the *Brassica carinata* group in the GEN RES CT99 109-112 project after 2 years

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Introduction

In the EU project "*Brassica* Collections for Broadening Agricultural Use, including Characterising and Utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop" (GEN RES CT99 109-112), the *Brassica carinata* subgroup is made up of the following partners:

- P15 - Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain (*sub-coordinator)* (IAS), and
- P16 - John Innes Centre, Norwich, United Kingdom (JIC).

Ethiopian mustard (*Brassica carinata* A. Braun) is an important crop within its natural environment (Ethiopia) and is increasingly being looked on as an additional oilseed crop with high potential for the dry areas of southern Europe where its drought tolerance and pest and disease resistance may make it suitable as an alternative oilseed within the crop rotation. Additionally, it contains many agronomic traits that would be beneficial if they could be incorporated within other *Brassica* crops grown in more temperate areas of Europe (Fereres *et al.* 1983; Anand *et al.* 1985; Gugel *et al.* 1990; Yitbarek 1992).

Role of the Brassica carinata *subgroup in the project*

The role of the *B. carinata* subgroup is to:

- Donate passport data of *B. carinata* germplasm held at IAS, Córdoba to the Bras-EDB;
- Characterize all available accessions using both molecular and phenotypic data;
- Create a *B. carinata* core collection;
- Regenerate the core collection; and
- Evaluate the core collection for characters of agronomic importance, including pest resistance and seed storage components.

Results achieved after two years

Updating the Bras-EDB with passport data

All the available information on passport data from the *B. carinata* accessions held at IAS has been sent for inclusion in the new version of the Bras-EDB. In relation to accessions obtained in the 1980s from Prof. Knowles (Dept. of Agronomy, University of California-Davis), the only information available at this moment is the original code numbers that Prof. Knowles provided with the accessions.

B. carinata core collection

In the start-up meeting it was decided that in the first year 100 accessions would be grown in field plots both at Norwich (from March to September 2000) and at Córdoba (from November 2000 to June 2001). These would be considered to be the preliminary core collection of *B. carinata.* A new set of 100 accessions from the collections at CGN (Wageningen) and IAS (Córdoba) would be grown and studied at Norwich and Córdoba during the second year (2001 to 2002).

The initial core collection will be modified after analyzing the combined data from JIC and IAS in years 1 and 2 so that accessions showing novel variation in morphological, agronomic or seed storage characters will be included in the initial core collection. Further modification will occur by selecting for trait stability and higher levels of homogeneity as indicated by genetic analysis using microsatellite markers.

Characterization and regeneration

In the start-up meeting the minimum descriptors to be used were discussed, using the "Descriptors for *Brassica* and *Raphanus*" (IBPGR 1990) as reference. A wide range of traits related to plant morphology, seed storage characteristics and molecular information (microsatellite markers) were also considered. A definitive list with the minimum descriptors for *B. carinata* will be determined at the end of the project, after analysis of the data obtained at JIC and IAS, to ensure that stable characters are selected.

Selfed seeds from all the accessions grown in year 1, both at JIC and IAS, have been obtained. Additionally, 30 accessions have been regenerated in the field at Córdoba in single-row plots within nylon mesh cages to ensure self-pollination within the accession. Partner 1 of the project (Centre for Genetic Resources, the Netherlands) has also regenerated 2 accessions of *B. carinata* during spatial evaluation of *Triticale*.

Evaluation

Methods for evaluation of agromorphological, seed storage and molecular traits were agreed during the start-up meeting. All the 100 accessions of the initial core collection grown both at JIC and IAS are now fully evaluated for these traits.

A large degree of variability within and between accessions has been observed for agromorphological characters including flowering time, plant height, seed colour, seed weight and seed yield.

Near infrared reflectance spectroscopy (NIRS) calibrations were performed to evaluate the main seed storage components (oil, protein, acid detergent fibre, fatty acids and glucosinolates) on selfed seeds from all the accessions of the core (Font *et al.* 2002a, 2002b). An important range of variation has been found among accessions for oil, protein, fibre and fatty acids (Table 1).

Additionally, the initial core collection has been evaluated in field conditions for resistance to flea beetle (*Psyllioides chrysocephala*) at JIC, while sporadic attacks of stem weevils (*Ceuthorrynchus picitarsis*) and cabbage aphid (*Brevicorne brassicae*) have been detected and recorded in some accessions at IAS.

Genetic analysis by using microsatellite markers has allowed us to observe a high degree of heterogeneity within accessions, and only a few accessions appear to be uniform at >1 microsatellite locus (Chinoy *et al.* 2002).

Table 1. Mean and range of variation for seed storage components in the *B. carinata* core collection

Descriptor	Mean	Minimum	Maximum
1000-seed weight (g)	4.00	2.70	5.40
Seed weight/plant (g)	83.90	29.00	248.20
Protein (% DW)	24.30	18.20	31.70
Adf (% dw)	11.65	6.60	17.40
Oil content (% DW)	38.90	27.00	50.90
Oleic acid (% oil)	9.35	1.80	18.90
Linoleic acid (% oil)	16.60	10.00	23.10
Linolenic acid (% oil)	13.05	6.60	18.10
Erucic acid (% oil)	40.65	23.20	53.10

Summary (mid-term assessment)

All required milestones defined in the project Technical Annex for the first 2 years have been fulfilled. Important achievements of the *Brassica carinata* subgroup are:

- A preliminary core collection of *B. carinata* has been established, grown and studied at Norwich and Córdoba during years 2001 and 2002.
- Minimum descriptors including morphological, seed storage and molecular traits were agreed, and characterization and evaluation started in accordance with the milestones of the project.
- All the accessions of the core were evaluated for agromorphological, seed storage and molecular traits, and for resistance to selected pests.
- The statistical analysis of all data obtained during these 2 years of field trials in two very different sets of environmental conditions (Norwich and Córdoba) will allow us to evaluate the stability of these traits, with special attention to the G x E effects on seed yield and seed quality characters.

Acknowledgement

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APPENDICES

Appendix I. Abbreviations and acronyms **76**
Appendix II. Agenda **77**
Appendix III. List of Participants **78**

Appendix I. Abbreviations and acronyms

AARI	Aegean Agricultural Research Institute, Izmir, Turkey
BAZ	Federal Centre for Breeding Research on Cultivated Plants, Braunschweig, Germany
Bras-EDB	*Brassica* European Database
CBD	Convention on Biological Diversity
CGN	Centre for Genetic Resources, the Netherlands, Wageningen, The Netherlands
CHM	Clearing House Mechanism
CMS	Cytoplasmic male sterility
DOFATA	Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, Catania, Italy
ECCDB	European Central Crop Database
ECP/GR	European Cooperative Programme for Crop Genetic Resources Networks
ENSA	Ecole nationale supérieure agronomique (France)
EPGRIS	European Plant Genetic Resources Information Infra-Structure
EU	European Union
EURISCO	European Internet Search Catalogue (EPGRIS project)
FAO	Food and Agriculture Organization of the United Nations, Italy
GGB	Greek Gene Bank, Thessaloniki, Greece
GIS	Geographic Information System
GRIN	Genetic Resources Information Network, USA
HRI	Horticulture Research International, Wellesbourne, UK
IAS	Instituto de Agricultura Sostenible, Córdoba, Spain
IDG	Istituto del Germoplasma, Bari, Italy
IHAR	Plant Breeding and Acclimatization Institute, Poland
INRA	Institut national de la recherche agronomique, France
IPGR	Institute of Plant Genetic Resources, Sadovo, Bulgaria
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany
IU	International Undertaking (FAO)
JIC	John Innes Centre, Norwich, United Kingdom
LIH	Lithuanian Institute of Horticulture
MBG	Mision Biologica de Galicia, Pontevedra, Spain
MCPD	Multi-crop Passport Descriptor List (FAO/IPGRI)
NCG	Network Coordinating Group (ECP/GR)
NGB	Nordic Gene Bank, Alnarp, Sweden
PGR	Plant genetic resources
RAPD	Random amplified polymorphic DNA
RICP	Research Institute of Crop Production, Prague, Czech Republic
RIFC	Research Institute of Fodder Crops, Troubsko, Czech Republic
RIOC	Research Institute of Oilseed Crops, Opava, Czech Republic
RIPP	Research Institute of Plant Production, Piešťany, Slovak Republic
SINGER	System-wide Information Network for Genetic Resources (CGIAR)
UPM	Universidad Politecnica de Madrid, Spain
UPOV	Union pour la protection des obtentions végétales (International Union for the Protection of New Varieties of Plants), Geneva, Switzerland
UPV	Universidad Politechnica de Valencia, Spain
USDA	United States Department of Agriculture
UTAD	University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
VIR	N.I. Vavilov Research Institute of Plant Industry, Russia

Appendix II. Agenda

Extraordinary Meeting of the ECP/GR Working Group on* Brassica, *held jointly with the Third Coordination Meeting of the EU Project GEN RES CT99 109-112

8-9 February 2002, Vila Real, Portugal

Friday 8 February

14:00 – 14:15 **Introduction**
- Opening of the meeting, welcome (*E. Rosa and G. Thomas)*

14:15 – 14:45 **ECP/GR**
- General briefing on ECP/GR *(L. Maggioni, 10 min)*
- Outcome of ECP/GR Steering Committee meeting in October 2001 *(L. Maggioni, 10 min)*

14:45 –15:00 **EPGRIS project**
- Implications for the *Brassica* WG *(E. Bettencourt, 15 min)*

15:00 – 16:00 **Review of data in the Central *Brassica* Database and provision for further data transfer** *(introduced by I. Boukema)*
- GIS analysis of the *Brassica* database (*L. Maggioni, 10 mi*n)

16:00 – 16:30 *Coffee break*

16:30 – 17:30 **Inclusion of characterization and evaluation data in the database**

17:30 –18:45 **Review of methodologies for safety-duplication and for regeneration**
- Regeneration activities carried out in Italy *(F. Branca, 10 min)*

Saturday 9 February

9:00 – 10:00 ***In situ* conservation actions** (*introduced by L. Maggioni*)

10:00 – 10:30 *Coffee break*

10:30 – 12:15 **Strengths and weaknesses in genetic resources work in each country**

12:15 – 12:30 **Any other business**

12:30 – 14:00 *Lunch*
14:00 – 18:00 *Free time except for the compilers of the draft report*

18:00 – 19:00 **Approval of workplan and recommendations**

19:00 – 19:15 **Closing remarks**

20:30 *Social dinner*

Appendix III. List of Participants

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