

Report of a Working Group on Cucurbits

First Meeting, 1-2 September 2005, Plovdiv, Bulgaria
M.J. Díez, W. van Dooijeweert, L. Maggioni and E. Lipman, *compilers*



European
Cooperative
Programme
for Plant
Genetic
Resources



ECP/GR

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Page 14: Bulgarian childrens' folk dance in front of the Institute for Plant Genetic Resources, Sadovo, Bulgaria, courtesy of @ L. Maggioni, Bioversity International.

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PART I. DISCUSSION AND RECOMMENDATIONS

Introduction

Opening of the meeting

Introductory welcome

Liliya Krasteva, Institute for Plant Genetic Resources "K. Malkov" (IPGR), Sadovo

Some of the greatest challenges of our century are the protection of nature and the conservation of biological diversity. With this in mind, it is my pleasure to open the First Meeting of the Working Group on Cucurbits of the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR)¹, which addresses these problems. I am very glad that the meeting is taking place here in Plovdiv. This is one of the most ancient cities not only in Bulgaria but also in Europe. Situated on the road connecting Europe and Asia, the city was well known as a big commercial and cultural centre in the past. There is one more special feature that distinguished the city from all the others, its unique location on seven hills on both sides of the Maritza River. There are also many historical monuments, museums and art galleries here. Ever since ancient times, cucurbit crops such as watermelons, melons, cucumbers and pumpkins have been introduced here. All of these crops have found favourable climatic conditions for their growth and for them the Plovdiv region became a second centre of origin. Finally, I would like to wish all of us a fruitful meeting and a pleasant time in Bulgaria.

Opening remarks

Lorenzo Maggioni, ECP/GR Coordinator, welcomed the participants to the First Meeting of the Working Group on Cucurbits and wished them a constructive meeting. He also complimented the local organizer Dr Liliya Krasteva, who recently replaced Dr Rada Koeva, both as Director of the Institute for Plant Genetic Resources, Sadovo and as ECP/GR National Coordinator for Bulgaria.

Finally, he thanked Maria José Díez and Willem van Dooijeweert for the technical preparations for the meeting which they undertook in their capacities of acting Chair and Vice-Chair of the Working Group (WG). He also suggested that M.J. Díez and W. van Dooijeweert could jointly chair this meeting. The Group agreed and M.J. Díez asked the participants to briefly introduce themselves.

The draft agenda was discussed and agreed by the Group (See Appendix V, pp. 62-63).

Briefing on ECP/GR Phase VII and other relevant PGR events in Europe

L. Maggioni gave an introduction to describe the current status of the ECP/GR Programme. He explained that the ECP/GR had entered its VIIth Phase (2004–2008) with some modifications made to the structure and mode of operation by the Steering Committee in its last meeting in Izmir, Turkey, October 2003.² With specific relevance for the WG on Cucurbits, it should be noticed that two Networks (Vegetables and Minor Crops) were

¹ Following the decision of the 10th meeting of the ECPGR Steering Committee in September 2006, the name of the Programme was simplified to "European Cooperative Programme for Plant Genetic Resources" and the acronym was also modified to "ECPGR", removing the traditional slash of "ECP/GR".

² See Report of the Ninth Steering Committee Meeting, also available on Internet (<http://www.ecpgr.cgiar.org/SteeringCommittee/SC9.htm>)

merged into a single one (Vegetables and Medicinal and Aromatic Plants Network), including seven WGs (*Allium*, *Brassica*, Cucurbits, Leafy Vegetables, Medicinal and Aromatic Plants, Solanaceae and Umbellifer Crops). The Steering Committee also requested a Network Coordinating Group (NCG) to define five priority WGs within the Network and to make proposals, in consultation with the WGs, for actions on the basis of a budget of about 200 000 euro allocated to the Network. As a result of this exercise, which went on during 2004, the WG on Cucurbits was included among the priority WGs for Phase VII. The following use of funds relevant for Cucurbits was eventually approved:

- September 2005: First Meeting of the Working Group on Cucurbits
- March 2006: Meeting of all Network Coordinating Groups
- 2007: Meeting of the Vegetables and Medicinal and Aromatic Plants Network (all seven WGs)³
- Publication of meeting reports
- Reserve funds for priority WGs (EU project preparatory meetings, data or sample acquisition, public awareness actions): 30 000 euro.

For further information on ECP/GR, the ECP/GR Web site was recommended, where several reference documents are available, including the Networks' budget and the Terms of Reference for the ECP/GR operational bodies. A specific Web page is also dedicated to the WG on Cucurbits and this can be improved with the help of WG members and according to the needs of the WG.

A briefing was then given about the recently launched first call for submission of project proposals under EC Regulation 870/2004, with a deadline 30 September 2005. Several WGs were preparing proposals, ranging from the further development of the European Plant Genetic Resources (PGR) information system (*ex situ* and *in situ* data) to the characterization of several specific crops (Leafy vegetables, Maize, Oats, Umbellifers, *Vitis*, etc.).

The future development of the European Internet Search Catalogue (EURISCO) and the overall mechanism of data flow in Europe will be relevant for this WG as well. Although for the moment it is recommended that the specific Central Crop Databases, such as the European Central Cucurbits Database (ECCUDB), should continue to be developed in the traditional way (i.e. by gathering all the available data from any available sources), in the medium term, it should become possible to download all the necessary passport data from EURISCO and to focus the attention of the ECCUDB on characterization and evaluation data and on the analysis of the database in order to offer other services to the users of cucurbit genetic resources.

A short account was also given of the ECP/GR-funded project AEGIS (A European Genebank Integrated System), which is planning, through a feasibility study, to promote the creation of a rational European PGR genebank system of genetically unique and important accessions, in order to conserve them safely in the long term, at the same time ensuring their genetic integrity, viability and availability to users. More information on AEGIS is available from www.ecpgr.cgiar.org/AEGIS/AEGIS_home.htm.⁴

³ As of September 2006, the renamed "Vegetables Network" only includes six Working Groups, with exclusion of the Medicinal and Aromatic Plants WG, that was included in the Sugar, Starch and Fibre Crops Network.

⁴ The Strategic Framework discussion paper was finalized in February 2008: ECPGR. 2008. A Strategic Framework for the Implementation of a European Genebank Integrated System (AEGIS). European Cooperative Programme for Plant Genetic Resources (ECPGR). Bioversity International, Rome, Italy. (available at http://www.ecpgr.cgiar.org/AEGIS/Docs/AEGIS_DiscussionPaper.pdf)

Acting Chairperson's report and outline of activities of the Working Group on Cucurbits

Presented by Maria José Díez

Introduction

The Working Group on Cucurbits was established following an initiative by the Vegetables Network Coordinating Group. A meeting of the Vegetables Network Coordinating Group was held in Portugal in 2000. The aim of the Network was to increase networking activities by increasing the number of Working Groups, which until then consisted of the *Allium*, *Brassica* and Umbellifer Crops WGs. One of the results of this meeting was the establishment of three additional Informal Working Groups (IWGs) for Solanaceae, Cucurbits and Leafy Vegetables. Focal persons were nominated with the responsibility of establishing workplans for the respective IWGs, developing crop databases and deciding the possible goals in terms of regeneration standards, establishment of minimum descriptor lists for each crop, safety-duplication and rationalization of the collections, and to organize meetings jointly with the ongoing EC-funded GENRES projects. The responsibility for cucurbits was accepted by the Institute for Conservation and Improvement of Valencian Agrodiversity (Centro de Conservación y Mejora de la Agrodiversidad Valenciana, COMAV) in the Polytechnic University of Valencia, Spain.

The first meeting of the IWG on Cucurbits was held in Adana (Turkey) in 2000,⁵ jointly with the annual meeting of the project GENRES CT99-108 on *Cucumis melo*. Representatives from Bulgaria, the Czech Republic, Hungary, the Netherlands, the Russian Federation, Spain and Turkey attended the meeting, as well as the EU *Cucumis melo* project partners from Portugal, Spain and Turkey. The mode of operation and the workplan of the IWG was defined, and consisted of the following tasks:

- Development of the European Central Cucurbits Database (ECCUDB);
- Assessment of the safety-duplication status of collections and planning of the transfer of safety-duplicates to genebanks with long-term conservation facilities;
- Development of regeneration guidelines and primary characterization protocols for cucurbitaceous species;
- Presentation of a letter to the next meeting of the Steering Committee asking for the formal establishment of the Working Group on Cucurbits.

Current status of the Working Group on Cucurbits

The first activity of the WG was the establishment of the European Central Cucurbits Database (ECCUDB), located at the Polytechnic University of Valencia (Spain) (<http://www.comav.upv.es/eccudb.html>). Currently the ECCUDB holds passport data of 22815 accessions stored in European institutions, 8905 of which correspond to the genus *Cucumis*, 6236 to *Cucurbita* and 5663 to *Citrullus*.

The proposal to establish a formal ECP/GR WG on Cucurbits as part of the Vegetables and Medicinal and Aromatic Plants Network was approved by the ECP/GR Steering Committee in October 2003. Currently 18 countries (Albania, Bulgaria, the Czech Republic, Georgia, Germany, Hungary, Ireland, Italy, Lithuania, the Netherlands, Poland, Portugal, Romania, Serbia and Montenegro, Slovakia, Slovenia, Spain and Turkey) have nominated their representatives to the WG on Cucurbits (contact details available at http://www.biodiversityinternational.org/networks/ecpgr/contacts/ecpgr_wgcu.asp).

The workplan of the WG was established in 2002 and reviewed and updated in 2004.

⁵ Díez, M.J., B. Picó and F. Nuez, compilers. 2002. Cucurbit genetic resources in Europe. *Ad hoc* meeting, 19 January 2002, Adana, Turkey. International Plant Genetic Resources Institute, Rome, Italy.

A proposal to be presented to the EU Genetic Resources Programme is being prepared and will be submitted for funding under EC Regulation 870/2004, following the AGRI GENRES 2005 call for proposals with a deadline 30 September 2005. The objectives of this action will strengthen and amplify the objectives proposed in the Cucurbits WG (see also p. 13).

Regeneration guidelines and primary characterization protocols for melon, cucumber, watermelon and *Cucurbita* species have been developed and will be discussed at the first meeting of the WG.

Reports on the status of national collections

Reports from countries not covered by the Adana report (2002)

Germany

Presented by Maria José Díez, on behalf of Bärbel Schmidt (full paper pp. 20-24)

The cucurbit collection of the German genebank in Gatersleben comprises a large collection of domesticated plants as well as their wild relatives. A total of 2702 different accessions of more than 20 genera are included; they originate from all over the world. The seeds are stored at -15°C. Herbarium specimens and photographs of the fruits are also available.

Italy

Presented by Nadia Ficcadenti (full paper pp. 25-28)

Melon is the main cucurbit crop in Italy. Data on cucurbit collections in Italy were gathered from breeders' collections, specifically from Agricultural Experimental Institutes (Monsampolo del Tronto and Montanaso Lombardo), the University of Bari, University of Palermo and the Italian National Agency for New Technologies, Energy and the Environment (Ente per le Nuove Tecnologie, l'Energia e l'Ambiente, ENEA), Roma. However, no passport data for these breeders' collections were available and no data were provided from the main Italian genebank in Bari. N. Ficcadenti gave an account of 294 accessions (240 melon) of local origin and 45 foreign accessions (37 melon). Descriptors used for evaluation are morphological, biochemical, biological, agronomic, disease resistance and molecular markers.

Considering that no passport data from Italy are included in the ECCUDB, M.J. Díez asked N. Ficcadenti to try if possible to facilitate the collection of Italian passport data for inclusion into the central database.

Poland

Presented by Katarzyna Niemirowicz-Szczytt, also on behalf of Teresa Kotlińska (full paper pp. 31-35)

Only five Cucurbitaceae species can be cultivated in Poland: *Cucumis sativus* (the most important in Poland), *C. melo*, *Cucurbita maxima*, *C. pepo* and *Citrullus vulgaris*. The aim of the national genebank is to try to collect all material that is tolerant to low temperature and material that is relatively early, because of the short period for plant growth in Poland. The Research Institute for Vegetable Crops (RIVC) in Skierniewice is responsible for the national vegetable genetic resources programme. Each year, part of the collection is regenerated and characterized at the Department of Plant Genetics, University of Warsaw. Seeds are then deposited in the National Centre for PGR in Radzików, conserved at 0°C or -15°C in glass jars. A total of 872 accessions of Cucurbitaceae of various origins are conserved in the Polish Gene Bank. Cucurbit accessions are not duplicated. The Department of Plant Genetics also maintains a collection of 22 mutants of cucumber and a collection of polyploids. These are

not available for distribution. There are some taxonomic uncertainties in the collection which need to be resolved. Open days are organized, such as the melon day, with the purpose of popularizing melon, which is not very well known in Poland. Students help to maintain the collection and in this way they learn about it. Passport data are collected in the FAO/IPGRI *Multi-crop Passport Descriptors* (MCPD)⁶ format. The material is generally available, when there is enough seed. Collecting missions are often organized to collect from home gardens in Poland.

M.J. Díez observed that passport data from Poland are not included in the ECCUDB and K. Niemirowicz-Szczytt offered to contact T. Kotlińska to make sure that the data can be transferred.

Serbia and Montenegro

Janos Berenji was unable to attend. A short report on the status of the cucurbit collections in Serbia and Montenegro was provided after the meeting and is included in Part II (p. 37).

Slovenia

Janko Verbić was unable to attend.

Short update on other national collections

Bulgaria

Presented by Liliya Krasteva

The Bulgarian cucurbit collection includes 1378 accessions, mainly of *Cucumis sativus*. They are conserved in long-term storage at -18°C or short-term storage at $+6^{\circ}\text{C}$ in the genebank.

A total of 500 accessions of melon, 320 of watermelon and 160 of *Cucurbita* spp. have been evaluated for resistance to diseases in the laboratory as well as in the open field.

About 50 accessions of each species are regenerated annually. The accessions are evaluated according to the descriptor list of the International Union for the Protection of New Varieties of Plants (Union internationale pour la Protection des Obtentions Végétales, UPOV). Electrophoresis has been used to assess a total of 30 accessions of *Cucumis melo*.

At present the enlargement of the collection is one of the main aims at IPGR-Sadovo and each year the collection increases by 20-30 accessions, mostly through free exchange between national and international institutions. During the last few years there have been no collecting missions, owing to lack of finance. Our efforts are directed towards the resumption of these activities and also to the development of on-farm conservation.

Czech Republic

Presented by Kateřina Karlová (full paper pp. 17-19)

About 9000 vegetable accessions and 530 medicinal plants are conserved in Olomouc. Among these, about 1800 are cucurbit accessions, with *Cucumis sativus* as the most important, followed by *Cucurbita* spp. and *Cucumis melo*.

Regeneration is the main goal for the genebank at this time, since several accessions need regeneration and characterization. However, there is no intention of enlarging the collection before regeneration has been completed. Only 80 accessions can be regenerated each year. About 150 accessions were distributed to users around the world during the last three years. Research activities are also planned, such as the analysis of carotenoid content in pumpkin

⁶ http://www.biodiversityinternational.org/publications/pubfile.asp?id_pub=124

and testing for virus infection. This year, exhibitions of pumpkins are being organized at the Prague Botanical Garden and at the open-air museum in Rožnov.

Hungary

Presented by Lajos Horváth

The Institute for Agrobotany in Tápiószele (ABI)⁷ conserves 1604 accessions (including 516 of *Cucurbita pepo*, 321 *C. maxima*, 209 *Cucumis melo*, 218 *Citrullus lanatus*), including several exotic species. Half of the accessions have been collected in Hungary or in the Carpathian valley. For the field multiplication a set of 20-30 space-isolated nurseries (with minimum 150 m distance) is used. Conservation is carried out according to international standards. Most of the cucurbit collection is duplicated within ABI.

Four different descriptor lists are used for characterization and 90% of the collection has been characterized. The passport documentation of the collection is complete and data are available from the Internet (national inventory), from EURISCO and from the ECCUDB. A new documentation system will be ready this year. All samples of the ABI genebank are freely available to users. Recently, a comparative seed DNA analysis of 57 modern *Cucumis melo* varieties was made, in comparison with a seed sample from the 15th century found at a historical site in Budapest. One specific *inodorus* variety was found to be the most similar to the ancient sample.

The Netherlands

Presented by Willem van Dooijeweert (full paper pp. 29-30)

An ISO 9001 quality management system was adopted by CGN (Centre for Genetic Resources, the Netherlands) in 2005. The collection has about 1000 accessions of *Cucumis sativus* only. Almost the whole collection was regenerated in six years with the help of breeders. Characterization was carried out during regeneration and all the data are available on-line.

Evaluation will predominantly be made by breeding companies and the data will be received back from them. A major update was made of passport data, with 500 fields changed out of 33 000 fields reviewed. A small melon collection of 65 accessions was received, including some accessions of old Dutch origin. CGN is involved in a Dutch project proposal aiming to introduce different and forgotten types of cucumber for the organic market.

Portugal

Presented by Valdemar Carnide (full paper p. 36)

The main collection is in the national genebank in Braga, with 532 accessions (mainly *Cucurbita*). There is another relevant collection in Vila Real (Universidade de Trás-os-Montes e Alto Douro). In total 591 accessions of national origin are conserved. The origins of the melon, cucumber and *Cucurbita* accessions are mainly from the North of Portugal. Part of the collection has been characterized for morphological or molecular markers, as a result of specific breeding projects. There are plans to increase the collection with samples from the South of Portugal.

Spain

Presented by Maria José Díez (full paper pp. 38-41)

The vegetable collection at national level is coordinated by COMAV, Valencia, with funds from the National Institute for Agricultural Research, Madrid. There are four collections in

⁷ As of January 2007, the Institute has been taken over by the Central Agricultural Office, Budapest, and is now the Research Centre for Agrobotany.

Spain: the Vegetable Genebank in Zaragoza; the Centre for Genetic Resources, Madrid; the Experimental Station "La Mayora", Málaga; and COMAV, Valencia, which is an active genebank conserving seed in glass jars (1855 accessions of the main species). Almost all of this collection is safety-duplicated in Madrid and Zaragoza. Samples are freely available. COMAV's evaluation activities aim at studying interactions with viruses and fungi. Results are published in printed catalogues.

Turkey

Presented by Sevgi Mutlu

The Turkish cucurbit collection is conserved at the Aegean Agricultural Research Institute (AARI) and safety-duplicated in Ankara. It consists of about 2000 accessions. Long-term storage is at -20°C, medium-term at 0°C and short-term at +4°C. Regeneration is made in isolation cages when viability falls below 60%. Characterization of snake melon was completed in 2004 and melon and watermelon are being studied in PhD projects. Other projects are planned for further characterization.

Mode of operation: discussion of the workplan of the Working Group on Cucurbits and its schedule

Introduction

M.J. Díez introduced the workplan for the WG, as approved in May 2003 during the Vegetables Network meeting⁸, and asked for comments and suggestions to update or revise the workplan.

W. van Dooijeweert suggested adding in the workplan an item on how to deal with taxonomy, since several accessions remain unidentified in several collections, due to the difficulties of taxonomic determination. This is confirmed by the analysis of the central database, which shows that more than 900 accessions are not yet taxonomically determined.

He also stressed the importance of collecting or managing landraces where they still exist.

Current status of the European Central Cucurbits Database (ECCUDB)

Presented by M.J. Díez

The European Central Cucurbits Database is held at the Institute for Conservation and Improvement of Valencian Agrobiodiversity (COMAV) at the Polytechnic University of Valencia (Spain). It is accessible through the ECPGR Web page (<http://www.ecpgr.cgiar.org/Databases/databases.htm>) or through COMAV's Web page (<http://www.comav.upv.es/eccudb.html>). The database format follows the FAO/IPGRI *Multi-crop Passport Descriptor List*. It currently holds 22 815 cucurbit passport data from 37 institutions belonging to 21 countries. These include 20 804 passport data corresponding to the cultivated species (5663 *Citrullus lanatus*, 2988 *Cucumis melo*, 5917 *Cucumis sativus* and 6236 of the main cultivated species of the genus *Cucurbita*, *C. maxima*, *C. pepo*, *C. moschata* and *C. ficifolia*). This represents 91% of the total number of accessions, the rest being wild species and unclassified samples. Nine institutions hold more than 500 accessions, representing over 93% of the total conserved in European collections. The main collection is that of the N.I. Vavilov Research Institute for Plant Industry (VIR, St. Petersburg, Russian Federation)

⁸ Thomas, G., D. Astley, I. Boukema, M.C. Daunay, A. Del Greco, M.J. Díez, W. van Dooijeweert, J. Keller, T. Kotlińska, A. Lebeda, E. Lipman, L. Maggioni and E. Rosa, compilers. 2005. Report of a Vegetables Network. Joint Meeting with an *ad hoc* group on Leafy Vegetables, 22-24 May 2003, Skierniewice, Poland. International Plant Genetic Resources Institute, Rome, Italy.

with 9975 accessions of cucurbit crops. There are accessions originating from more than 100 countries for the main genera *Cucumis*, *Citrullus* and *Cucurbita*.

The ECCUDB is searchable on-line. It allows searches on 21 fields by filling in the search form. The results page shows a simplified set of six fields out of the complete set. A button labelled "details" allows the user to reveal the complete available data set for an accession. Question marks show the meaning of codes when the user positions the cursor over them. The information contained in the database can be completely downloaded. Information about contributors is also available.

The structure of the ECCUDB will be modified to include the characterization and evaluation data agreed by the WG on Cucurbits for melon, cucumber, watermelon and *Cucurbita* species. Information on taxonomy of cucurbits will also be included.

Updating passport data at CGN

Presented by W. van Dooijeweert

Beside uploading passport data into the ECP/GR Cucurbits database, the importance of the quality of these data was discussed. Passport data are of great importance for the effective use and management of germplasm collections. Users of genebank material make choices mainly based upon passport data and curators use passport data to group accessions, to build core collections and to identify redundancy and gaps in their collections. Finally, passport data play a major role in the determination of the most significant original material, in order to allow the removal of redundant seed samples among genebanks.

In a PowerPoint presentation W. van Dooijeweert showed the work done in the last few years at CGN, concerning the updating of passport data of the fruit vegetable collections. The result of this update was the finding that many fields were empty and that data could be added after a thorough check. Also, many fields were filled but the information was not correct because of misinterpretations of field definitions.

A parallel was made with data in other collections and the concern was expressed that a lot of data in the ECP/GR databases are far from complete and sometimes even incorrect. Reliability and quality of data is the starting point for an optimal cooperation of genebanks on an international scale, the intention of the ECP/GR Working Groups.

Examples were given of fields which were also misinterpreted in the ECP/GR Cucurbits and Solanaceae databases. Fields describing the origin of an accession are often filled incorrectly. In the CGN passport descriptors "Origin country" and "Origin address", the country and address of the donor are often given instead.

Partners were asked to pay attention to the passport data of their own collections, since improvements in these will result in higher quality of their own database and also in a higher quality of the ECP/GR databases. Attention should also be paid to the use of the FAO/IPGRI *Multi-crop Passport Descriptor List* when data are submitted for uploading onto the ECP/GR database. It is possible to upload in an efficient way only if the MCPDs are followed strictly.

Planning for safety-duplication of each collection under long-term conservation conditions

(Introduced by W. van Dooijeweert)

Taking into consideration that several new partners joined the WG on Cucurbits after the *ad hoc* meeting held in Adana, Turkey in 2002, the current level of safety-duplication and the availability of WG members to host black boxes⁹ was discussed again. W. van Dooijeweert

⁹ Under "black box" arrangement, the safety-duplicate seed sample is stored in long-term conditions according to international standards; it is not used, tested, regenerated or distributed to a third party.

stressed the importance of having safety-duplicates by quoting three examples that he had encountered in the last few years:

1. Eggplant material collected in the 1980s held in the University of Birmingham died, but it had been safety-duplicated at CGN and was therefore saved.
2. Due to war in Sierra Leone, a lot of material was lost, but it was also conserved in CGN and can now be re-sent to Sierra Leone.
3. Wild potato material collected in Bolivia in the 1980s was eventually lost there, but CGN maintained it and it can now be replaced in Bolivia.

The table produced in the report of the meeting in Adana was reviewed. All partners were asked the same three questions:

- What is the current level of safety-duplication?
- Which holding has long-term conservation facilities?
- Who would be available to host safety-duplicates as “black boxes”?

The same question was asked of the members of the Group who were not present at the meeting. The result of this survey is included in Table 1.

Table 1. Current level of safety-duplication of cucurbit collections in Europe

Holding	Safety-duplication	Long-term conservation facilities	Availability to host black boxes
Institute for Plant Genetic Resources “K. Malkov” (IPGR), Bulgaria	100% safety-duplication only within the institute	Yes	Yes
Research Institute of Crop Production (RICP), Czech Republic	30% (but 100% of the regenerated material)	Yes	Yes, under bilateral agreement, but only in limited amounts, depending on the sample size
Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany ¹⁰	100%	Yes	Yes, depending on available space
Institute for Agrobotany (ABI), Hungary	66% duplicated only within the same institution	Yes	No
Experimental Institute Monsampolo del Tronto, Italy	No – can consider sending duplicates	No	No
Centre for Genetic Resources, Wageningen, The Netherlands (CGN)	100%	Yes	Yes
National Plant Genetic Resources Centre, Plant Breeding and Acclimatization Institute (IHAR), Radzików, Poland	Currently not duplicated	Yes	Yes
Banco Português de Germoplasma Vegetal (BPGV), Braga, Portugal	No – under discussion	Yes	Yes
N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russian Federation	80%	Yes	No
Centro de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universidad Politécnica de Valencia, Spain	100%	No	No
Experimental Station “La Mayora”, Consejo Superior de Investigaciones Científicas (CSIC), Málaga, Spain	75%	No	No
Aegean Agricultural Research Institute (AARI), Izmir, Turkey	100%	Yes	No
Çukurova University, Adana, Turkey	Planned	No	No

¹⁰ Now Leibniz Institute of Plant Genetics and Crop Plant Research

Whenever agreements are made between two institutions to establish safety-duplicates under black box arrangements, it is recommended that a memorandum of understanding should be signed by the two parties. This document should indicate the responsibilities of both parties, the type and quality of material to be duplicated and guidelines for packing the material.¹¹

Comments made by the partners on their situations as regards safety-duplication:

- Bulgaria: safety-duplication only within the genebank. Will consider sending safety-duplicates to a different genebank under a black box arrangement.
- Czech Republic: all safety-duplicated twice, in Piešťany and Prague.
- Hungary: safety-duplication of 2/3 of the material, but only inside the same institute. At present there are no funds to complete safety-duplication or to send the material to a different genebank under a black box agreement.
- Italy: since there are different collection holders in Italy, the situation is not clear and will be checked.
- The Netherlands: no changes in the way safety-duplicates are handled.
- Spain: all safety-duplicated in Madrid and Zaragoza.
- Turkey: the collection is safety-duplicated in the genebank in Ankara.

The appropriate number of seeds per accession for the black box was discussed. It should be enough to ensure a successful regeneration. In CGN, the regeneration package for cucumber is set at 100 seeds.

The need for minimum descriptor lists

(Introduced by W. van Doonjeweert)

Each collection holder is currently using a different set of descriptors for characterization. This will become a problem when one tries to combine the data from all holdings into one database.

In order to provide information through a central database, such as the ECCUDB, there is a need to harmonize the data by agreeing on a minimum descriptor list for each crop.

This can be solved through an agreement on how to put the data together, without any need to change the system that each curator has traditionally been using. Only data gathered using the minimum lists will be incorporated in the database.

Establishment of minimum descriptor lists for each crop

A few items were raised for discussion and the conclusions are summarized below:

- Leaf size (and other descriptors) might need references. However, reference varieties grow differently in different environments. Alternatively, a scale might be given, and this option was preferred.
- Colour charts vary in the different institutes and even the same chart under different luminosity gives a different impression. It should be possible to define dark and light green by comparison of the various accessions in a plot. This is what is needed for a minimum description, which is not aiming at perfect definition of the colour.

¹¹ A sample of bilateral agreement for safety-duplication under black box arrangement, established between the Nordic Gene Bank and the Institute of Biology of Salaspils, Latvia, is available as Annex I, pp. 101-102 *in*: Maggioni, L. and T. Gass. 1998. Safety-duplication of germplasm collections in Europe. Pp. 96-102 *in* Report of a Working Group on Forages. Sixth meeting, 6-8 March 1997, Beitostølen, Norway. International Plant Genetic Resources Institute, Rome, Italy.

- In general it was agreed that the minimum descriptors should give only an indication about the morphology of an accession, therefore they should not be too detailed.

A first draft of the agreed minimum descriptors for *Cucumis melo*, *Cucumis sativus*, *Citrullus lanatus* and *Cucurbita* spp. was distributed to all the participants during the meeting. These lists have been submitted to IPGRI¹² for revision and approval.¹³

Establishment of a regeneration protocol for cucurbits

The Group discussed the importance of having general guidelines for regeneration. It was agreed to prepare general guidelines that will be valid for all cucurbit crops. These guidelines contain only general practices for good regeneration and give some specific examples. After intense discussion, the first draft was produced and sent to all WG partners for their comments before finalization. The final version is included as Appendix II (pp. 57-58).

Information on identification of cucurbit material on the Internet

M.J. Díez informed the Group about the possibility of finding Internet sources of information on cucurbit taxonomy, including images that can be useful for the identification of accessions. Other sources can help in clarifying the species authorities. Additionally, holding institutions, genetic resources accessions, bibliographic sources and lists of genes can also be found.

A list of useful Web addresses is included as Appendix I (p. 56).

Discussion on the most relevant problems of cucurbit cultivation in each country

Main pathogen problems in the cultivation of melons in Spain

Presented by Maria José Díez

The major pests of melon crops in Spain are the two-spotted spider mite (*Tetranychus urticae* Koch), the western flower thrips (*Frankliniella occidentalis* Pergande), two different whiteflies (*Bemisia tabaci* Genadius and *Trialeurodes vaporariorum* West), aphids (*Myzus persicae* Sulzer and *Aphis gossypii* Glover), and leafminers (*Liriomyza* spp.). These are very harmful not only as pests, but also as vectors for different viral diseases. The most significant viruses in the open field are those transmitted by aphids, such as the cucumber mosaic virus (CMV), the watermelon mosaic virus (WMV-2), the zucchini yellowing mosaic virus (ZYMV) and the papaya ringspot virus (PRSV). In glasshouse-grown melons, the most significant viruses are the melon necrotic spot virus (MNSV), which is transmitted by the soil-borne fungus

¹² With effect from 1 December 2006, IPGRI and INIBAP operate under the name "Bioversity International", Bioversity for short. This new name echoes their new strategy, which focuses on improving people's lives through biodiversity research.

¹³ The descriptors were reviewed during the parallel meeting of the Cucurbits WG held during the second meeting of the Vegetables Network in Olomouc, Czech Republic, June 2007 (see: Report of a Vegetables Network. Second Meeting, 26–28 June 2007, Olomouc, Czech Republic [<http://www.ecpgr.cgiar.org/Workgroups/Cucurbits/Cucurbits.htm>]). Minor modifications were made. The finalized version of the descriptors will be uploaded on the WG's Web page (<http://www.ecpgr.cgiar.org/Workgroups/Cucurbits/Cucurbits.htm>).

Ospidium bornovanus, the cucumber vein yellowing virus (CVYV) and the cucumber yellow stunting disorder virus (CYSDV), which are both transmitted by *B. tabaci*.

Beside viral diseases, widespread fungal diseases affect melons in Spain, such as powdery mildew (*Sphaerotheca fuliginea*, syn. *S. fusca*), soil-borne fungal diseases such as fusarium wilt, which is caused by *Fusarium oxysporum* f.sp. *melonis*, and the melon vine decline, also referred to as melon dieback, collapse or sudden wilt, which causes plant death. This syndrome, which has been associated with various pathogens, causes the greatest losses in melon cultivation in Spain (see paper on "Breeding melons for resistance to melon vine decline" in Part II, pp. 43-47).

W. van Dooijeweert mentioned the problem that escapes from cultivation might be wrongly considered as landraces.

Other scientific and technical contribution

Assessing procedures for optimal regeneration of Cucurbita spp. at COMAV

Presented by Maria José Díez (full paper pp. 48-54)

Efficient *ex situ* conservation relies on maintaining the genetic integrity of the original accessions. Losses of genetic diversity are associated with both the frequency of regeneration and the regeneration methods used. In each regeneration cycle, the genetic integrity of each accession is threatened by contamination and genetic erosion. Contamination is strongly dependent on the breeding system, which determines the risk of outcrossing. *Cucurbita* species are assumed to be entomophilous and open-pollinated and the use of insect-proof cages is particularly useful to avoid alien pollen contamination. However, cages are quite expensive and insect management is tedious and costly. Therefore, in many institutions, the regeneration of *Cucurbita* species is performed by flower bagging and hand-pollination under greenhouse or open-air conditions. Various hand-pollination methods are commonly applied, and the regenerated samples usually consist of seed mixtures from self- and cross-pollinated fruits. A series of studies aimed at establishing guidelines for regeneration of species of *Cucurbita* have been carried out. Self-pollination, controlled cross-pollination and uncontrolled pollination methods were used. The number of fruits set, the number of seeds per fruit and the seed germination percentages obtained with different pollination methods were determined. Our preliminary results show the efficiency of hand-pollination methods in regenerating *Cucurbita* accessions as an alternative to insect-pollination using isolation cages. In terms of fruit set, number of seeds per fruit, and germination rates, these methods are successful for *Cucurbita* regeneration. However, hand-pollination is labour-intensive, especially for selfing methods in which each plant has to be selfed to avoid the loss of genetic diversity. Other methods such as pollination with pollen mixtures, which can reduce the number of pollinations, thus ensuring the preservation of genetic diversity, are currently being assessed.

The way ahead

Opportunities for funding the Working Group's activities (EC programmes)

Following the publication in July 2005 of the call for proposals under Council Regulation (EC) 870/2004, a project proposal is being prepared, with the title: "Stimulating the use of cucurbit genetic resources in Europe".¹⁴

The objectives of the project will be to complete the ECCUDB, establish a core collection, arrange for safety-duplication, implement regeneration, characterization and evaluation of the collections, promote *in situ* and on-farm conservation and disseminate the results.

All the main cucurbit crops in Europe (melon, cucumber, watermelon and *Cucurbita* species (*C. maxima*, *C. pepo*, *C. moschata*, *C. ficifolia*)) will be included in the project. Partners will be the following:

- **Genebanks**
CGN, Wageningen, The Netherlands
IPK, Gatersleben, Germany
- **Genebanks associated with breeding institutes**
COMAV, Valencia, Spain (project coordinator)
IGV, Bari, Italy
- **Breeding institutes**
Çukurova University, Adana, Turkey
INRA, Montfavet, France
EPSO, Alicante, Spain
UTAD, Vila Real, Portugal
SIA, Zaragoza, Spain
CSIC, Málaga, Spain
- **Seed companies**
Plantum NL, AL Gouda, The Netherlands
SEMINIS, Murcia, Spain
Western Seeds, Spain
- **Non-governmental organizations**
Dreschflegel, Witzenhausen, Germany

Among non-EU partners, VIR (Russian Federation) will participate in the meetings and provide access to their collection.

Benefits are expected for the implementation of the WG workplan as a result of the interaction between the project and the WG:

- Experience derived from the EU project will be exploited by the members of the WG;
- The database will be updated and completed;
- Regeneration protocols and minimum descriptor lists will be discussed and reviewed; Modifications can be integrated by the WG;
- Core collections will be created.

¹⁴ Update at time of publication: the proposal was submitted to the first and second calls of the AGRI GEN RES Community Programme of the European Commission, Council Regulation (EC) 870/2004. Unfortunately the proposal was not funded.

Perspectives for the future of the Working Group on Cucurbits

A new workplan was discussed and endorsed by the Group (see Appendix III, p. 59).

The next meeting of the WG is planned to take place in 2007, during the Vegetables Network meeting.

Conclusion

Presentation of the report and adoption of recommendations

A draft report was distributed to all the participants, discussed, and endorsed by the Group with minor modifications.

Selection of the Working Group Chair and Vice-Chair

The Group suggested that M.J. Díez and W. van Dooijeweert be elected as Chair and Vice-Chair respectively and they kindly accepted this commitment.

Closing remarks

The Group visited the Institute for Plant Genetic Resources “K. Malkov” (IPGR), Sadovo in the morning of 2 September and had the opportunity to see the botanic garden and the national genebank. The Group was also delighted to attend at a Bulgarian folk dance performed by school children (photos below) and to taste traditional dishes prepared with pumpkins. A visit was also organized to the Bashkovo Monastery.

All the staff of IPGR Sadovo were thanked for their warm hospitality and for the perfect organization of the meeting.



PART II. PRESENTATIONS AND PAPERS

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National reports

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Status of the national cucurbitaceous vegetables collection in the Czech Republic

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Introduction

The Czech National Gene Bank belongs to the Research Institute of Crop Production (RICP)¹⁵ in Prague-Ruzyne since 1994, but the collecting of vegetable genetic resources had already started around 1920 at the Moravian Institute of Agricultural Research in Brno, and was continued later on at the Research Institute of Vegetable Growing and Breeding in Olomouc. Since 1994, when the Ministry of Agriculture of the Czech Republic accepted the "National programme of conservation and utilization of genetic resources of cultivated plants", RICP has been the national coordinator of this programme, and coordinates activities of ten private and/or state institutions which keep genetic resources of all plant species cultivated in our climatic conditions. The collections of vegetables and medicinal, aromatic and spice plants are held in the Gene Bank in Olomouc, a detached unit of RICP. More than 10 000 accessions of about 430 botanical species are held in Olomouc (Dušek 2004); the cucurbit collection, with 1829 accessions, is one of the largest.

Position of cucurbitaceous vegetables in Czech agriculture

Cultivation of cucurbit vegetables has a long tradition in the Czech Republic. Some species have been documented here since the Middle Ages and they continue to be very popular. However only cucumbers (*Cucumis sativus* L.) have an economic importance (Table 1).

Table 1. Production, market and consumption statistics for cucurbits in the Czech Republic (source: Buchtová 2004)

	Species	Year					
		1999	2000	2001	2002	2003	2004
Production area (ha)	<i>C. sativus</i> gr. Cucumber	1 190	1 142	831	448	364	85*
	<i>C. sativus</i> gr. Gherkin	2 792	2 705	2 201	1 236	987	302*
Yield (t)	<i>C. sativus</i> gr. Cucumber	17 862	15 122	10 766	8 811	10 456	2 621*
	<i>C. sativus</i> gr. Gherkin	34 246	25 535	24 575	25 812	18 594	5 062*
Average yield (t/ha)	<i>C. sativus</i> gr. Cucumber	15.02	13.25	19.95	19.67	28.71	30.78*
	<i>C. sativus</i> gr. Gherkin	12.27	9.44	11.17	20.88	18.85	16.76*
Import (t)	<i>C. sativus</i> gr. Cucumber			42 821	48 136	44 267	23 346 [†]
	<i>C. sativus</i> gr. Gherkin			6 614	7 394	6 145	6 559 [†]
	<i>C. melo</i> + <i>C. lanatus</i>			58 916	75 414	79 417	44 065 [†]
Export (t)	<i>C. sativus</i> gr. Cucumber			242	381	289	1 632 [†]
	<i>C. sativus</i> gr. Gherkin			0.5	46	196	283 [†]
	<i>C. melo</i> + <i>C. lanatus</i>			81	181	86	2 691 [†]
Consumption (kg/head)	<i>C. sativus</i> gr. Cucumber	6.6	6.1	5.5	5.7	5.5*	
	<i>C. sativus</i> gr. Gherkin	3.8	2.9	3.1	3.3	2.7*	
	<i>C. melo</i> + <i>C. lanatus</i>	8.8	7.4	6.9	6.6	6.1*	

* Estimation of the Czech Statistical Institute

[†] Period 1 January–30 September 2004

¹⁵ As of 1 January 2007, RICP was renamed Crop Research Institute (CRI).

Most of the gherkin crop is processed as pickles. Pumpkins (*Cucurbita maxima* Duch.) as well as squashes (*C. pepo* L.) are cultivated mainly in hobby gardens. Melons (*Cucumis melo* L.) and watermelons (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) can be grown only in the warmest regions and they are grown in private gardens.

The cucurbit collection, regeneration and safety-duplication

An overview of cucurbit genetic resources collection and of the current status of regeneration and multiplication at the end of August 2005 is given in Table 2. Passport data of all accessions are available on-line (<http://genbank.vurv.cz/genetic/resources/default.htm>).

Table 2. Structure of the Czech cucurbit collection and its regeneration

	No. of accessions	Accessions regenerated (%)
<i>Cucumis sativus</i>	801	42
<i>Cucumis melo</i>	104	28
<i>Cucumis</i> (other spp.)	85	29
<i>Cucurbita</i> spp.	782	18
<i>Benincasa hispida</i>	6	0
<i>Citrullus lanatus</i>	8	25
<i>Echinocystis</i> spp.	1	0
<i>Lagenaria</i> spp.	12	25
<i>Luffa</i> spp.	16	0
<i>Momordica balsamina</i>	3	0
<i>Trichosanthes</i> spp.	11	0
Total	1829	

The collection contains predominantly landraces, local cultivars and older cultivars; 28 wild *Cucumis* species and 27 wild *Cucurbita* species are also represented: however, the taxonomic identity should be verified for accessions of both genera. Even after revision of the collection some duplicates are probably still present and the duplicates should be eliminated after detailed study of their morphological and if possible also biochemical characters.

As shown in Table 2, only about 30% of accessions have already been regenerated. The regeneration and multiplication of seeds is done in isolation cages (2.3 x 5.5 m) covered by glass or plastic nets; plants are pollinated by honey bees or bumble bees. The technical capacity for regeneration is about 80 accessions each year.

After regeneration (according to international standards in both active and base collections) the seeds are dried to 5-6% moisture content, placed in hermetically closed jars and stored at about -18°C in the main seed store of the Gene Bank of RICP in Prague. A small sample of seeds is also kept in Olomouc as a working collection. The most important accessions, consisting predominantly of Czech (Czechoslovak) original cultivars and landraces are also sent to the Gene Bank in Piešťany (Slovak Republic), where, according to the agreement between representatives of the Czech Republic and Slovakia, the safety-duplicates are stored.

Characterization and evaluation

The *Cucumis* and *Cucurbita* collections are characterized and evaluated in successive steps using the minimum list of descriptors developed by E. Křístková (*in* Chytilová *et al.* 2004). For each crop ten characters were selected:

- for *Cucumis sativus* L.: plant growth habit, length/diameter ratio of fruit, fruit cervix, fruit shape at stem end, spine colour of young fruit, young fruit – type of spines, predominating skin colour of young fruit, predominating skin colour of fruit at botanical maturity, parthenocarpy and strategy of reproduction

- for cultivated species of *Cucurbita* genus (*C. maxima*, *C. pepo*, *C. moschata*, *C. ficifolia* and *C. argyrosperma*): plant growth habit, leaf slit depth, leaf shape, fruit shape, predominant fruit skin colour, secondary fruit skin colour, peduncle transectional shape, peduncle type at the connection with the fruit, seed coat colour and seed margin colour.

Collection enrichment and utilization

The enlargement of the collection is not a priority at the moment. Only 4 new *Cucurbita* spp. accessions have been entered since 2003, while 175 *Cucurbita* accessions were distributed to Czech users (universities, breeding companies, etc.) and also abroad.

Collaboration, research and other activities

Collaboration between the Gene Bank in Olomouc and the Palacký University in Olomouc has been very important in the past and it is now planned to extend this collaboration to the Mendel University of Agriculture and Forestry in Brno. Plans for the future include the study of carotenoid content in pumpkins and testing for virus infections.

The cucurbit germplasm collection was demonstrated to the public at the Prague Botanical Garden in Prague-Troja (1–30 September 2005) and also in the open-air museum in Rožnov pod Radhoštěm (15–30 October 2005). Participation in similar projects for the general public is also planned.

Acknowledgments

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Cucurbits in the German ex situ genebank in Gatersleben

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Status of the collection

The *ex situ* cucurbit collection of the German genebank consists of 2702 different accessions belonging to more than 20 genera. The collection contains cultivars and domesticated plants as well as their wild relatives. Each year around 130 accessions are under cultivation to reproduce fresh seed material. The taxonomic composition and the number of accessions which were grown in 2005 are shown in Table 1.

Table 1. Status of the Cucurbitaceae collection in the German genebank

Cucurbitaceae	No. of accessions	No. of accessions under cultivation (in 2005)
<i>Benincasa</i>	13	0
<i>Citrullus colocynthis</i>	12	3
<i>Citrullus lanatus</i>	246	17
<i>Coccinia</i>	1	0
<i>Cucumis melo</i>	453	19
<i>Cucumis sativus</i>	625	21
<i>Cucumis</i> spp.	77	10
<i>Cucurbita ficifolia</i>	9	0
<i>Cucurbita maxima</i>	349	13
<i>Cucurbita mixta</i>	17	1
<i>Cucurbita moschata</i>	103	0
<i>Cucurbita pepo</i>	559	17
<i>Cucurbita</i> spp.	87	4
<i>Cyclanthera</i>	7	2
<i>Diplocyclos</i>	3	0
<i>Ecballium</i>	2	1
<i>Echinocystis</i>	1	0
<i>Gynostemma</i>	1	1
<i>Kedrostis</i>	2	0
<i>Lagenaria</i>	73	17
<i>Luffa</i>	24	3
<i>Melothria</i>	6	0
<i>Momordica</i>	15	1
<i>Praecitrullus</i>	1	0
<i>Sechium</i>	6	0
<i>Thladiantha</i>	1	1
<i>Trichosanthes</i>	9	0
Total	2702	131

The countries of origin for the major species (more than 100 accessions) are listed in Table 2.

Table 2. Geographical origin of the major cucurbit species held in the German genebank

Country of origin*	<i>Citrullus lanatus</i>	<i>Cucumis melo</i>	<i>Cucumis sativus</i>	<i>Cucurbita maxima</i>	<i>Cucurbita moschata</i>	<i>Cucurbita pepo</i>
AFG	-	3	5	-	-	-
ALB	11	21	9	2	4	14
ARG	-	-	-	7	-	-
ARM	3	-	1	-	-	-
AUS	1	-	-	1	1	3
AUT	-	-	-	1	-	3
AZE	-	-	1	-	-	-
BGR	17	11	22	5	-	6
BRA	1	2	1	-	-	1
CAN	1	2	-	-	-	3
CHE	-	-	3	-	-	-
CHL	-	2	-	3	-	1
CHN	6	8	23	2	3	3
COL	-	-	-	-	2	1
CZE	-	1	-	2	-	2
CUB	-	3	14	-	9	-
DEU	-	29	37	2	-	26
DNK	-	1	2	-	-	-
EGY	2	1	-	-	1	2
ESP	-	7	5	1	-	1
ETH	-	-	-	2	-	-
FIN	-	-	1	-	-	-
FRA	1	6	10	2	-	-
GBR	-	1	2	-	-	2
GEO	12	26	40	44	3	76
GRC	4	14	1	-	-	9
GTM	-	1	-	-	-	1
HRV	4	7	9	6	1	18
HUN	5	9	4	8	-	8
IND	-	-	6	-	4	-
IRL	1	-	-	-	-	-
IRQ	2	15	-	8	1	2
IRN	-	-	1	12	1	-
ISR	3	9	-	-	-	4
ITA	25	73	23	17	14	83
JPN	6	16	63	2	3	3
COG	1	-	-	-	-	-
KAZ	-	-	1	-	-	-
KOR	-	-	5	-	1	-
LBY	1	13	1	1	-	2
MAR	-	-	-	-	1	-
MDA	-	-	2	-	-	-
MEX	2	1	-	1	1	3
MKD	-	-	-	-	-	1
MNG	4	2	3	1	-	2
NER	-	-	-	1	-	-
NLD	-	-	18	-	1	-
NGA	-	1	-	-	-	1
NPL	-	-	-	-	1	-
PER	-	-	-	3	-	-
POL	-	-	2	-	-	4
PRK	3	-	8	5	8	3
PRT	4	1	-	-	-	-
PYF	-	-	-	-	1	-
ROM	1	-	6	16	-	24
RUS	8	21	37	2	1	5
SEN	-	2	-	-	-	-
SLV	-	-	-	-	2	-
SUN	4	16	34	4	1	12
SVK	-	-	1	-	-	3
SVN	-	-	-	-	-	2

* ISO country codes

Table 2 (cont.). Geographical origin of the major cucurbit species held in the German genebank

Country of origin*	<i>Citrullus lanatus</i>	<i>Cucumis melo</i>	<i>Cucumis sativus</i>	<i>Cucurbita maxima</i>	<i>Cucurbita moschata</i>	<i>Cucurbita pepo</i>
SWE	-	6	2	-	-	6
SYR	-	-	1	1	-	-
TJK	4	3	3	1	1	-
TKM	-	1	-	-	-	-
TUN	3	11	-	2	3	3
TUR	6	7	10	46	2	6
UKR	8	-	5	-	-	3
URY	-	-	-	1	-	-
USA	50	67	21	19	4	30
UZB	-	10	3	5	2	5
VNM	-	1	-	-	-	-
YEM	2	-	-	-	-	1
YUG	3	1	3	3	-	4
ZAF	6	2	1	5	-	2
ZIM	1	-	-	-	-	-
ZMB	1	-	-	-	1	-
Unknown	29	19	175	105	25	165

* ISO country codes

- ***Citrullus lanatus* (Thunb.) Matsum. et Nakai**

A total of 246 accessions from nearly all over the world are available. Most of the accessions are from USA (50) and from Italy (25).

- ***Cucumis melo* L.**

The collection of *Cucumis melo* originates from 42 different countries. Most of the accessions are again from USA and Italy. A lot of accessions are from expeditions to Russia and Georgia.

- ***Cucumis sativus* L.**

Many accessions are from expeditions to East European countries, e.g. 40 accessions from Georgia and 22 from Bulgaria. Most of the material is from agricultural institutes in Japan.

- ***Cucurbita maxima* Duchesne**

Again a couple of accessions are from expeditions to Georgia. The others are from many different countries.

- ***Cucurbita moschata* Duchesne**

Moschata accessions are from 30 different countries of origin.

- ***Cucurbita pepo* L.**

Accessions are available from expeditions to Georgia and Italy but also from agricultural institutes from the USA.

Fig. 1 gives an overview of the regional origins of the major cucurbits held in the German genebank.

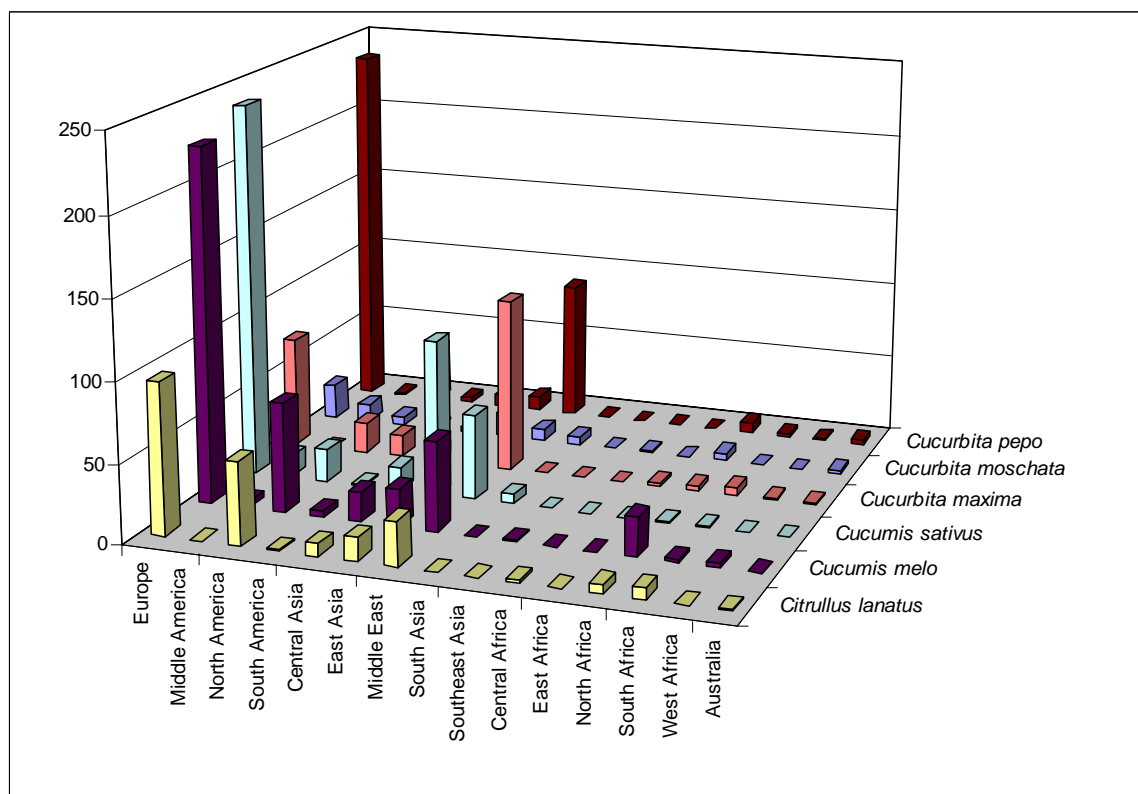


Fig. 1. Regional origins of the major cucurbit species held in the German genebank.

Characterization

In order to describe the accessions it is necessary to have a practical characterization system. Very detailed descriptors are available for Cucurbitaceae (Esquinas-Alcazar and Gulick 1983) and for melon (*Cucumis melo* L.) (IPGRI 2003). UPOV has also published *Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability* for cucumber (1996), pumpkin (1996), squash (2002), watermelon (2004) and melon (2006) (UPOV Web site, http://www.upov.int/en/publications/tg_rom/tg_index_numerical.html).

For the German genebank material it is not possible to evaluate all the characters mentioned in these descriptors. In order to get a precise description of the accessions an adapted version useful for characterization in the field and in the greenhouse was developed. The adapted descriptors include all the important characters necessary for a distinct classification of the accessions and for the taxonomic determination of the genebank material.

Taxonomy

For the taxonomic classification two books are available with good keys for determination of genera and species (Whitaker and Davis 1962; Kirkbride 1993). The monograph of the genus *Cucumis* (Kirkbride 1993) is also available as an interactive key on the Internet (<http://nt.ars-grin.gov/sbmlweb/OnlineResources/Cucumis/Index.cfm>). Furthermore, some interesting papers from various journals are also helpful for the final determination (Grebenscikov 1950, 1958, 1969; Hammer *et al.* 1986; Stepansky *et al.* 1999; Pitrat *et al.* 2000; Křístková *et al.* 2003; Teppner 2004).

Conservation¹⁶

All accessions are conserved in a cold storage facility at -15°C . The safety-duplicates are stored in aluminium bags under vacuum at the Svalbard Global Seed Vault. At regular intervals germination tests are performed to find out the germination percentage of the collection's accessions.

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¹⁶ Updated March 2008

Genetic resources of Cucurbitaceae in Italy

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Importance of cucurbit cultivation in Italy

Cucurbits are important crops which are cultivated in almost all Italian regions (Figs. 1 and 2) where the environmental conditions are suitable for growth as spring forcing crops (melon, zucchini, watermelon and cucumber), as semi-late and open-field or under-tunnel crops and also as winter crops under greenhouses (zucchini, cucumber and melon) (Ficcadenti and Crinò 1998). Cucurbits represent a precious reservoir of genetic material for geneticists and horticulturalists and moreover they are commonly used in the diet as sources of carbohydrates, pumpkin and squash in cooked dishes, watermelon and melon as fruit, cucumber as pickled gherkins.

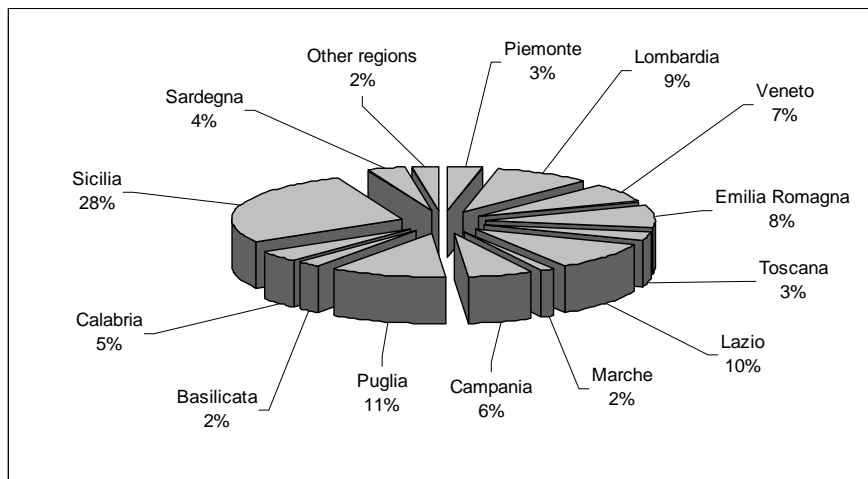


Fig. 1. Percentage distribution of cucurbit crops in the Italian regions on a total area of 59 871 ha.

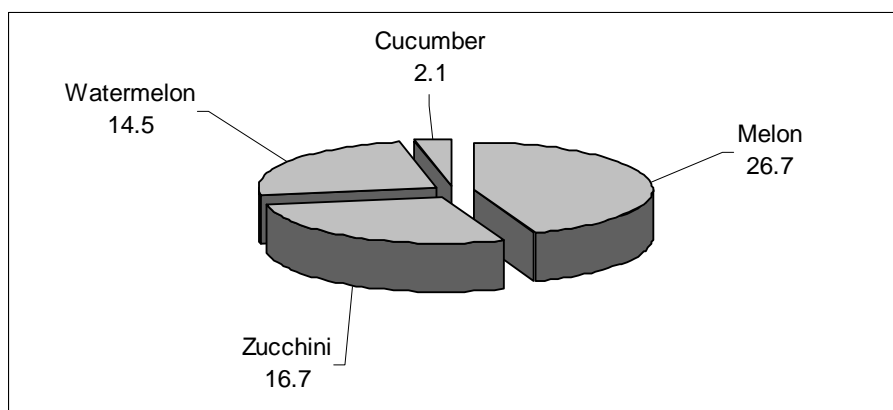


Fig. 2. Harvested area (x 1000 ha) of each main cucurbit crop in the Italian peninsula.

- **Melon (*Cucumis melo* L.)**

Melon made its first appearance in Italy during the period of the Roman Empire, as illustrated in a painting found near Naples. In Italy 26 626 ha of melons are cultivated with an average yield of 22.6 t/ha for a total production of 601 758 t (ISTAT 2004). In the last 50 years, the covered area has increased due to a greater market demand and the diffusion of the “protected” cropping method (Ficcadenti 1998).

There are three important botanical varieties of cultivated melon: *reticulatus*, *cantalupensis* and *inodorus*.

“Summer” types (botanical varieties *reticulatus* and *cantalupensis*) are grown on 68% of the cultivated area, primarily in the central and northern regions of Italy. The F1 hybrids of the *reticulatus* varieties make up more than 80% of the production. There is some cultivation of *cantalupensis* while the *inodorus* “winter types” are grown on 32% of the cultivated area, mostly in the south and on the islands using such populations as ‘Giallo di Paceco’, ‘Purceddu’, ‘Rugoso di Cosenza’, ‘Napoletano giallo’, etc. At present only a few new *inodorus* F1 hybrids, bred by various foreign seed companies, are being cultivated. Sicily is the most important producer with 10 640 ha of which 9743 ha are cultivated almost exclusively in the open air.

The Italian breeding programme aims at:

1. improving several pure lines of *reticulatus*, *cantalupensis* and *inodorus* botanical varieties
2. introducing genes for resistance to the main diseases
3. making the best possible use of melon landraces.

- **Zucchini (*Cucurbita pepo* L.)**

Zucchini is cultivated in almost all Italian regions (16 653 ha, ISTAT 2004) and consequently, several local cultivars are able to supply the local markets. The consumer prefers types with long and striated fruits (‘Striata d’Italia’, ‘Striata di Napoli’, etc.) in southern Italy and oval or round fruit and long and dark or light green fruit (‘Bolognese’, ‘Tonda di Piacenza’, etc.) in central Italy. It is still quite difficult to substitute the old cultivars with both the improved ones and F1 hybrids, often imported cultivars. Zucchini is particularly widespread in south-central Italy where 12 167 ha, mainly concentrated in Sicily, Puglia, Lazio, Calabria and Campania are cultivated. The demand for out-of-season products also favours the cultivation of the species under greenhouse conditions.

Zucchini plants are severely affected by viral infections, particularly those caused by watermelon mosaic virus (WMV1 and WMV2), zucchini yellowing mosaic virus (ZYMV), cucumber mosaic virus (CMV) and squash mosaic virus (SqMV). The viruses are leading to a decrease of zucchini acreages in Italy, and the introduction of resistance genes into the plants is the main objective of zucchini breeding.

- **Watermelon (*Citrullus vulgaris* L.)**

In Italy, watermelon is cultivated on 14 516 ha with a production of 587 392 t (ISTAT 2004). A general decrease of both the acreage and production in the traditional growing regions as well as a decline in exports underlines a quite critical situation for this species. Many foreign hybrids, which are replacing the large number of local varieties such as ‘Precoce di Romagna’, ‘Quarantina’, ‘Comacchiese’ or ‘Brindisina’, etc. are now cultivated because of their better uniformity of fruit size and their organoleptic traits. Few breeding programmes were developed in the past. Currently, studies are focused on: (i) obtaining information on the heredity of gene transmission; (ii) recognizing the most interesting cultivars as sources of useful traits for breeding purposes; (iii) estimating the advantages of hybrids compared to the open-pollinated varieties; and (iv) addressing the breeding programme by exploiting both the Italian genetic material and the foreign germplasm.

- **Cucumber (*Cucumis sativus* L.)**

In the last 30 years an increased request for out-of-season products by both national and foreign markets has led to an expansion of Italian cucumber cultivation. However, ISTAT data of the last ten years (ISTAT 1994) show a decrease in production and acreages, with present values of 76 867 t and 2075 ha most of which are for fresh consumption (ISTAT 2004). Lazio and Puglia are the most important regions producing cucumber; they are followed by Sicilia, Veneto and Emilia Romagna. Foreign varieties and F1 hybrids are not well adapted to the pedoclimatic conditions of our country, therefore Italian landraces such as 'Lungo di Chioggia', 'Mezzo lungo di Chioggia', 'Verde lunghissimo', 'Lungo verde degli ortolani', etc. are still cultivated. Breeding activities are mainly focused on stabilizing the gynoecious character in order to obtain parent material to build F1 hybrids. Parthenocarpy, freedom from bitterness and disease resistance characters are the other important goals for the breeding programme.

The Italian cucurbit collection

- **Composition of the collection**

The status of the Italian cucurbit collection is given in Table 1.

Table 1. Number of accessions collected of each cucurbit species

Genus	Species	Botanical type	No. of accessions	
			Foreign	Local
<i>Cucumis</i>	<i>melo</i>	<i>inodorus</i>		118
		<i>reticulatus</i>		17
		<i>cantalupensis</i>		36
		<i>conomon</i>		5
		<i>adzhur</i> ('Caroselli')		33
		'Barattieri' (unknown)		31
	<i>ficifolius</i>		1	
	<i>africanus</i>		1	
	<i>metuliferus</i>		32	
	<i>anguria</i>		2	
<i>zeyhery</i>		1		
Total <i>Cucumis</i>			37	240
<i>Cucurbita</i>	<i>moschata</i>		2	
	<i>figari</i>		1	
	<i>maxima</i>			3
	<i>pepo</i>			5
Total <i>Cucurbita</i>			3	8
Other genera				
<i>Citrullus</i>	<i>colocynthis</i>		1	
<i>Lagenaria</i>	<i>siceraria</i>		1	
<i>Benincasa</i>	<i>cerifera</i>		2	
<i>Ecballium</i>	<i>elaterium</i>		1	
<i>Luffa</i>	<i>cilindrica</i>			1
Total other genera			5	1
Total Cucurbits			45	249

The national collection comprises 294 accessions including 45 introduced and 249 local forms. The collections consist mainly of 240 accessions of *Cucumis melo*, 37 of *Cucumis* spp., 11 of *Cucurbita* spp. and 6 of others species. The national cucurbit collection is conserved in the holding institutions by breeder collections as shown in Table 2.

- **Conservation and safety-duplication**

All accessions in the collection are conserved in the holding institutions and safety-duplicated by breeders.

Table 2. Status of cucurbit germplasm collections in Italy and their curators

Breeder collections	<i>Cucumis</i> spp.	<i>Cucurbita</i> spp.	Others*	Total
N. Ficcadenti Research Institute for Vegetable Crops Section of Monsampolo del Tronto (AP)	33	-	3	36
V. Ferrari Research Institute for Vegetable Crops Section of Monsampolo del Tronto (AP)	15	8	-	23
L. Ricciardi University of Bari Section of Genetics and Breeding	153	-	-	153
P. Crinò ENEA, UTS Biotec, C.R. Casaccia, Rome	36	3	3	42
M. Schiavi Research Institute for Vegetable Crops Section of Montanaso Lombardo (LO)	3	-	-	3
V.V. Bianco University of Bari Department of Crop Production Sciences	25	-	-	25
G. Incalcaterra University of Palermo Section of Horticulture and Floriculture	12	-	-	12
Total	277	11	6	294

* Other cucurbit species (*Benincasa*, *Citrullus*, *Ecballium*, *Lagenaria*, *Luffa*)

References

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Status of the CGN cucurbit collection – an update

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Introduction

Since the last overview of the CGN cucurbit collection (van Dooijeweert 2002), a few changes have been made. They will be described in this update.

CGN has focused on a limited number of collections, for which it attempts to maintain high quality seed, which is readily available to *bona fide* users. To ensure the quality of our work CGN has adopted a quality management system which has been externally certified according to ISO9001:2000, and most of its operations have been certified since December 2004 by RWTÜV, a major German certification body. The English version of the Quality Manual describing organization and procedures is available on CGN's Web site (<http://www.cgn.wur.nl>).

The collection

The cucumber collection has been extended. Now 934 accessions are available to the public (Table 1). In 2000 and 2001 a major update was done on the passport data. This update resulted in major changes. More than 33 000 fields were checked and reviewed. About 2500 fields were filled with new data and 500 fields were changed because the information was not correct. Also a small number of fields were emptied because the information was not correct.

This result indicates that the quality of the passport data can be improved. By improving passport data of collection holders, the quality of the ECPGR Cucurbits Database will be improved. For rationalization it is necessary that passport data are correct. Only then will it be possible to identify gaps or duplication.

Table 1. Number of *Cucumis* accessions per taxon in the CGN collection

Botanic name	No. of accessions
<i>Cucumis sativus</i> group unknown	216
<i>Cucumis sativus</i> group Cucumber	365
<i>Cucumis sativus</i> group Gherkin	352
<i>Cucumis sativus</i> var. <i>hardwickii</i>	1
Total	934

Since 2005 a small melon collection has been added. This collection is a small working collection of the former Institute for Horticultural Plant Breeding (IVT) and holds 65 accessions. It consists of a few old Dutch varieties and some *C. melo* var. *flexuosus* (snake melon) accessions. The viability of the material has not been tested yet and all accessions need regeneration.

Utilization

To enhance utilization of the cucumber collection we improved the search facilities. Twenty plant, fruit and flowering characteristics of cucumber have been made searchable on-line. It is also possible to download all passport, characterization and evaluation data. After the lettuce and the potato collection, the "core selection" technology is now also available for the cucumber collection as well. This technology allows on-line selection of a set of "most diverse" accessions meeting the criteria of your search.

Currently (September 2005) a proposal for a new project is being developed to introduce unfamiliar or forgotten types of cucumber in the market. These types must be produced by organic farmers.

Reference

Dooijeweert, W. van. 2002. Status of the cucumber (*Cucumis sativus*) collection of CGN. Pp. 33-35 in Cucurbit genetic resources in Europe. *Ad hoc* meeting, 19 January 2002, Adana, Turkey (M.J. Díez, B. Picó and F. Nuez, compilers). International Plant Genetic Resources Institute, Rome, Italy.

The Cucurbitaceae germplasm collection in Poland

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Of the 30 cucurbit species cultivated all over the world, 5 are successfully grown in Poland, both in open field and in greenhouses. Poland is the northernmost European country where these species are encountered under field conditions and therefore provides an excellent opportunity to test the plants for earliness and cold tolerance. A favourable circumstance is that in this kind of moderate climate pests and diseases are not as frequent as in southern Europe.

The Plant Genetic Resources Laboratory of the Research Institute of Vegetable Crops (RIVC), Skierniewice, is responsible for the conservation of the national vegetable genetic resource collection (including cucurbits), which is part of the national genebank.

During the past 12 years as many as 872 accessions representing 5 species were reproduced and evaluated. The current status, type of accessions and geographical origin of the cucurbit germplasm is given in Tables 1 and 2.

Every year a working collection of selected cucurbit species is grown out at the Department of Plant Genetics, Breeding and Biotechnology of Warsaw Agricultural University (WAU). Taxonomic identification, multiplication and evaluation of the accessions collected during collecting missions are carried out, as well as regeneration of the accessions deposited in the genebank store if necessary.

Table 1. Status of the Cucurbitaceae collection in the Polish Gene Bank, RIVC, Skierniewice, 2005

Species	English / Polish names	No. of accessions					Distributed within country 2003-2005
		Total	With passport data	With evaluation/ characterization data	Seed in long-term storage	Regene-rated 2003-2004	
<i>Cucumis sativus</i> L.	Cucumber / Ogórek	449	426	218	426	34	165
<i>Cucumis melo</i> L.	Melon / Melon	88	88	40	88	20	14
<i>Citrullus lanatus</i> (Thunb.) Matsum. et Nakai	Watermelon / Arbuz	57	57	46	57	10	4
<i>Cucurbita pepo</i> L. and <i>C. maxima</i> Duch. ex Lam.	Pumpkin / Dynia	222	222	131	222	24	65
<i>Cucurbita pepo</i> L. convar. <i>giromontina</i>	Zucchini / Cukinia	30	30	21	30	4	7
<i>Cucurbita pepo</i> L. convar. <i>patissonina</i>	Pattypan squash / Patison	10	10	8	10	4	7
<i>Cucurbita pepo</i> L. convar. <i>giromontina</i>	Squash / Kabaczek	11	11	11	11		2
Other cucurbits		5	5	1	5		1
Total		872	849	476	849	96	265

Table 2. Type of sample and origin of the cucurbit accessions in the Polish Gene Bank, RIVC, Skierniewice, 2005

Species	No. of accessions					Per country of origin (ISO country codes)
	Total	Advanced cultivar	Breeding materials	Landrace	Wild	
<i>Cucumis sativus</i> L.	449	97	42	309	1	EGY - 1, CAN - 1, CHN - 12, DEU - 1, IND - 1, ISR - 1, ITA - 1, JPN - 14, KOR - 4, MDA - 2, NLD - 7, ROM - 3, RUS - 29, TJK - 10, UZB - 1, UKR - 44, USA - 22, LVA - 2, POL - 293
<i>Cucumis melo</i> L.	88	36	14	38		UKR - 7, CAN - 1, CHN - 13, FRA - 2, GEO - 2, POL - 12, MDA - 2, ALB - 7, USA - 4, TKM - 3, UZB - 11, SYR - 1, JPN - 2, RUS - 2, YUG - 4, Unknown origin - 15
<i>Citrullus lanatus</i> (Thunb.) Matsum. et Nakai	57	26		31		ALB - 4, EGY - 3, MDA - 3, POL - 6, TKM - 2, UKR - 4, USA - 13, UZB - 2, CHN - 10, KOR - 1, AFG - 1, RUS - 5, IND - 1, PAK - 1, SYR - 1
<i>Cucurbita pepo</i> L. and <i>C. maxima</i> Duch. ex Lam.	222	5	8	209		ALB - 5, CHN - 4, MDA - 1, RUS - 2, UZB - 1, SVK - 6, UKR - 40, USA - 1, ROM - 8, GRC - 1, POL - 153
<i>Cucurbita pepo</i> L. convar. <i>giromontina</i>	30	3		27		CHN - 1, POL - 19, SVK - 1, UKR - 6, GRC - 1, EGY - 2
<i>Cucurbita pepo</i> L. convar. <i>patissonina</i>	10	2		8		POL - 9, UKR - 1
<i>Cucurbita pepo</i> L. convar. <i>giromontina</i>	11			11		POL - 4, CHN - 1, UKR - 5
Other cucurbits	5			5		CHN - 4, POL - 1
Total	872	169	64	638	1	

Storage, regeneration and characterization

The seeds of all collected accessions are deposited in the central genebank store located at the National Centre for Plant Genetic Resources, Radzików. Temperature in chambers: 0°C (active collection), +15°C (long-term storage); moisture content of seeds 5–7%. The seeds are kept under vacuum in glass jars.

During 2003-2004 seed regeneration was carried out for 96 accessions (Table 1).

Characterization and evaluation data are available for 476 accessions (Table 1). The characterization includes 15 traits for pumpkin, watermelon, pattypan squash and zucchini, and 20 traits for cucumber and melon.

Cucurbit accessions are not duplicated so far.

In addition to the standard collection we maintain two specific collections at the Department of Plant Genetics, Breeding and Biotechnology: the collection of mutants (22 gene mutants of cucumber) and the collection of polyploids (9 tetraploids, 1 pentaploid and 1 hexaploid of cucumber). These collections are not available for distribution. Publications related to these collections and to additional research are listed in the literature section.

Evaluation for usefulness depends on the species and possibilities and includes characterization of morphological characters, evaluation of marketable traits, disease resistance, stress tolerance, molecular characterization, etc.

On-farm conservation has been carried out for the past three years in five organic farms located in southern Poland on the basis of an agreement between the farmers and the Plant Genetic Resources Laboratory. The aim is the reintroduction to their places of origin of landraces of cucurbits and other vegetables collected in the past. The landraces and old cultivars at these farms are grown and utilized according to local methods. In addition regeneration, characterization and popularization (by means of green schools, farmers' organizations, a Web site) are carried out.

Documentation and availability

Passport data of the collected accessions are prepared in accordance with the FAO/IPGRI *Multi-crop Passport Descriptors* and FAO WIEWS Descriptors. All passport and evaluation data are computerized.

Most accessions are freely available if the number of seeds is sufficient. For breeding material now removed from the catalogue, written permission must be obtained from the donor breeder.

Accession-related information is available upon request, as email attachments, on CD-ROMs and floppy discs.

Collecting missions

Collecting missions are organized every year in several regions of Poland and neighbouring countries to collect indigenous germplasm threatened by extinction.

Five missions were organized in 2003-2004 in selected regions of Poland, resulting in a total of 238 collected accessions, including 33 of cucurbits (Table 3).

Table 3. Cucurbitaceae germplasm collected in Poland by the Polish Gene Bank, 2003-2004

Date	Area	No. of accessions collected					
		Cucumber	Water-melon	Squash	Pumpkin	Pattypan squash	Zucchini
Nov. 2003	Opatów	1	1	1		1	1
Dec. 2003	Małopolska	1			5		
Sept. 2004	Kurpie	11			7		2
Oct. 2004	Małopolska				2		
Oct. 2004	Karkonosze						
Total per crop		13	1	1	14	1	3
Grand total		33					

During expeditions, all available information (such as local growing systems, local methods of plant protection, utilization for consumption or as medicinal plants, etc.) is recorded. This information is useful to choose the proper places for reintroduction of old cultivars. Detailed records are also taken of the collecting site by means of the Global Positioning System (GPS).

The sources of collected materials are mostly local markets, home gardens and home seed stores in isolated villages, where elderly farmers still maintain local cultivars of various vegetables in small quantities for domestic use.

Each collected sample is divided into two parts: one part is added to the base collection; the other is used for multiplication and preliminary evaluation.

Utilization and research activities carried out on the collection

Between 2003 and 2005 a total of 265 seed samples of cucurbits were distributed to users in Poland (Table 1).

Accessions collected in the genebank are used in various research programmes carried out at the universities, institutes and breeding companies. The results of such studies are provided to the genebank to enrich the existing database. The Plant Genetic Resources Laboratory cooperates with breeding companies and agricultural universities in carrying out research work or breeding programmes on the plant species of interest. This cooperation covers seed regeneration, maintenance of field collections, evaluation of morphological and marketable characters, resistance to pathogens, etc. The genebank material is used in creative breeding programmes. This kind of cooperation is supported by genebank funds (provided by the Ministry of Agriculture) on the basis of special agreements.

Perspectives for the future

Cucurbits are an economically important group of vegetables in Poland. Cucumber is considered the most important species and is the only one reported in the statistical yearbooks. The significance of summer squash is increasing and vegetables such as zucchini, patty pan and spaghetti squash, although almost unknown some 30 years ago, are becoming more and more popular. The economic value of winter squash is also increasing; it is widely used as a raw material in the food processing industry.

Since the statistical figures are not available yet, the significance of the above-mentioned species can be inferred from the quantity of seeds sold annually and the number of species in the register. On the basis of the sale of seeds it can be calculated that in Poland melon and watermelon are cultivated on 200 ha each, and summer squash and winter squash on 700 ha each. The cultivation area of cucumber is much bigger and can be estimated at 2000 ha.

In this situation it seems necessary to provide constant monitoring and evaluation of these crops. The Department of Plant Genetics, Breeding and Biotechnology of Warsaw Agricultural University ensures favourable conditions for the maintenance of the cucurbit collections (greenhouses, plastic tunnels, storage facilities in the laboratory building, etc.). Moreover, the collection fulfils a didactic function to students who are taught how to evaluate and conserve germplasm collection and recognize plant diversity.

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Cucurbit genetic resources collections in Portugal

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Collections

Since the last report (Carnide 2002) several collecting missions have been carried out. The total number of accessions of cucurbits has now reached 591, including 145 of *Cucumis melo*, 53 of *C. sativus*, 32 of *Citrullus lanatus*, 350 of *Cucurbita* spp. and 11 of *Lagenaria siceraria* (Table 1).

Table 1. Number of accessions and characterization/evaluation by institution

Institution*	No. of accessions	No. of accessions with		No. of accessions evaluated
		morphological characterization	molecular characterization	
BPGV-Braga				
<i>Cucumis melo</i>	131	25	5	25
<i>Cucumis sativus</i>	53	-	-	-
<i>Citrullus lanatus</i>	32	32	32	32
<i>Cucurbita</i> spp.	305	55	-	-
<i>Lagenaria siceraria</i>	11	-	-	-
UTAD-Vila Real				
<i>C. melo</i>	12	12	12	12
<i>Cucurbita</i> spp.	45	36	36	36
EAN-Oeiras				
<i>C. melo</i>	2	2	2	2
Total	591	162	87	107

* BPGV: Banco Português de Germoplasma Vegetal
 UTAD: Universidade de Trás-os-Montes e Alto Douro
 EAN: Estação Agronómica Nacional

Characterization and evaluation

The total number of accessions characterized and evaluated is 356 (60.2%). In general the characterization and evaluation are associated with breeding programmes.

Conservation

The accessions of the different collections are conserved in good conditions (seeds stored between -18°C and -16°C depending on the institution).

Reference

Carnide, V. 2002. Genetic resources of Cucurbitaceae in Portugal. P. 36 in *Cucurbit genetic resources in Europe*. *Ad hoc* meeting, 19 January 2002, Adana, Turkey (M.J. Díez, B. Picó and F. Nuez, compilers). International Plant Genetic Resources Institute, Rome, Italy.

Status of Cucurbitaceae genetic resources in Serbia and Montenegro

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General status

There is no active and centralized genebank in Serbia and Montenegro. Genetic resources management is part of the activities of research institutes dealing with genetics, plant breeding and certified seed production of field and vegetable crops. In most cases the accessions are part of the breeding material aimed at breeding new commercial varieties. The accessions are held in cool storage (+5°C) and maintained under field conditions as necessary. The level of documentation accompanying the accessions is variable. None of the accessions are fully described according to the official descriptors. However, all accessions are accompanied by basic data such as the origin, plant type, fruit type, potential way of use, etc.

The collections

- **Centre for Vegetable Crops in Smederevska Palanka** (Web site: www.cvcsp.co.yu)

1. *Cucurbita maxima*: 5 domestic populations
2. *Cucurbita pepo*: 20 domestic populations and 5 commercial varieties
3. *Cucurbita moschata*: 1 domestic population and 1 commercial variety
4. *Citrullus lanatus*: 55 commercial varieties
5. *Cucumis melo*: 20 domestic populations and 12 commercial varieties
6. *Cucumis sativus*: 52 commercial varieties

Contact person: Mr Nenad Pavlovic (npavlovic@cvcsp.co.yu)

- **Institute of Field and Vegetable Crops in Novi Sad** (Web site: <http://www.ifvcns.co.yu>)

1. *Cucurbita pepo* (naked seeded oil pumpkin): 14 commercial varieties
2. *Cucurbita pepo* and *C. maxima* (ornamental gourds): 30 different forms
3. *Lagenaria siceraria*: 34 accessions of different fruit shape and size
4. *Sechium edule*: 1 accession *in situ*
5. *Citrullus lanatus*: 2 domestic populations and 5 commercial varieties

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The territory of Serbia and Montenegro is still an attractive region for exploration and collection of cucurbit landraces, primarily for *Cucurbita* spp., followed in significance by melon and watermelon. The rapid spread of commercial varieties of all representatives of the Cucurbitaceae family is a growing threat to landraces, pointing to the urgent need for a systematic effort to collect all that remains from the once amazing variability of cucurbit genetic resources in Serbia and Montenegro.

Cucurbit genetic resources collections in Spain

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Status at national level

The Programme for the Conservation and Utilization of Genetic Resources was created by the Ministry Order of 23 April 1993. This Order established the Centre for Genetic Resources (Centro de Recursos Fitogenéticos, CRF, Madrid), which acts as Centre for the conservation of seeds of base collections and Centre for documentation of Spanish genetic resources. Regarding vegetables, the collection's characterization and documentation activities have been coordinated by the Institute for Conservation and Improvement of Valencian Agrodiversity (Centro de Conservación y Mejora de la Agrodiversidad Valenciana, COMAV) since 1994, through a national project funded by the National Institute for Agriculture and Food Research and Technology (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA). Ten institutions participate in the recently renewed Project:

- COMAV, Universidad Politécnica de Valencia (UPV), Valencia
- Misión Biológica de Galicia–Consejo Superior de Investigaciones Científicas (CSIC), Galicia
- Servicio de Investigación Agroalimentaria (SIA), Zaragoza
- Centro de Mejora Agraria El Chaparrillo, Delegación Provincial de Agricultura, Ciudad Real
- Instituto Andaluz de Investigación, Formación Agraria, Pesca, Alimentación y de la Producción Ecológica, Junta de Andalucía, Sevilla
- Estación Sericícola, Consejería Agricultura, Agua y Medio Ambiente, Murcia
- Consejería Agricultura y Pesca, Almería
- Universidad Politécnica de Cartagena, Murcia
- Instituto de Agricultura Sostenible (IAS), CSIC, Córdoba
- Consejería de Agricultura y Medio Ambiente, Toledo

The institutions listed in Table 1 hold the most important cucurbit collections.

The cucurbit collections at COMAV

COMAV currently holds 2272 cucurbit accessions, mainly from Spain (Table 2). In terms of the areas explored for cucurbits, the most extensively collected Autonomous Communities are the Comunidad Valenciana, Canarias, Andalucía, Cataluña and Castilla-La Mancha. Many other Communities remain almost uncollected. However, accessions from these Communities exist in other Spanish collections.

Table 1. Number of cucurbit accessions held in the main Spanish collections

Institution	<i>C. lanatus</i>	<i>Citrullus wild</i> ¹	<i>C. melo</i>	<i>C. sativus</i>	<i>Cucumis wild</i> ²	<i>Cucumis spp.</i>	<i>C. maxima</i>	<i>C. pepo</i>	<i>C. moschata</i>	<i>C. ficifolia</i>	<i>Cucurbita spp.</i>	Minor cucurbits ³	Total
Vegetable Genebank, Servicio de Investigación Agroalimentaria, Zaragoza	373	-	1410	432	-	-	-	-	-	-	305	-	2520
Center for Genetic Resources, Madrid	144	-	611	26	-	81	-	-	-	-	-	59	921
Experimental Station "La Mayora", Málaga	-	-	531	-	29	-	-	-	-	-	-	-	560
COMAV, Valencia	276	8	657	136	57	-	292	313	272	94	84	83	2272
Total	793	8	3209	594	86	81	292	313	272	94	389	142	6273

¹ include 4 species² include 19 species³ include 37 species of 17 genera**Table 2.** COMAV cucurbit collections

Autonomous Community	<i>Cucumis melo</i>	<i>Cucumis sativus</i>	<i>Citrullus lanatus</i>	<i>Cucurbita ficifolia</i>	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. pepo</i>	<i>Lagenaria siceraria</i>	<i>Citrullus colocynthis</i>	<i>Cucurbita sp.</i>	Total
Andalucía	134	38	31	10	49	18	36	5	2	7	330
Aragón	9	11	6	2	5	-	11	3	-	-	47
Asturias	-	1	-	-	1	-	10	-	-	-	12
Baleares	20	-	3	2	8	18	15	3	-	1	70
Canarias	30	7	42	28	9	128	77	4	2	17	344
Cantabria	-	-	-	-	-	1	4	-	-	-	5
Castilla La Mancha	45	19	25	3	16	7	18	4	-	2	139
Castilla León	11	5	4	-	11	-	6	-	-	9	46
Cataluña	40	3	56	6	22	15	27	-	-	10	179
Comunidad Valenciana	170	32	50	21	77	33	71	23	-	16	493
Extremadura	53	3	14	1	9	4	9	2	-	1	96
La Rioja	-	-	1	-	2	-	-	-	-	-	3
Madrid	2	-	-	-	-	-	-	-	-	-	2
Murcia	48	8	5	2	10	3	3	3	-	1	83
Navarra	1	-	-	-	1	-	1	-	-	-	3
País Vasco	-	-	-	-	2	1	-	-	-	-	3
Total	563	127	237	75	222	228	288	47	4	64	1855

The COMAV Genebank is an active genebank. Seeds are stored at +3°C in cool chambers. Silica gel is used to dry the seeds. Safety-duplications of the collections exist in other Spanish genebanks. All passport and characterization data are computerized.

The COMAV holds the European Central Cucurbits Database, accessible on-line (<http://www.comav.upv.es/eccudb.html>).

A research project has been recently awarded to establish the core collection of the *Cucurbita* genus. Seeds are available to users except in cases where there is a need for regeneration. More details about the collections were given by Picó *et al.* (2002).

Evaluation activities

The COMAV evaluation activities aim at studying interactions with viral and fungal pathogens. Approximately 300 accessions have been evaluated with various objectives.

The response of many wild *Cucumis* and *C. melo* accessions against melon vine decline has been tested, both in field trials and under controlled inoculation conditions (Esteva and Nuez 1994; Iglesias *et al.* 2000; Dias *et al.* 2004). The ascomycete fungus *Monosporascus cannonballus* seems to be the most extended and virulent species involved in melon collapse. New technologies based on real-time polymerase chain reaction (PCR) are currently used to quantitatively detect the pathogen in melon roots (Pico *et al.* 2005). These accessions are also being evaluated by the structure of their root system, as a vigorous and deeply penetrating root system may contribute to increase the tolerance to this and other biotic and abiotic soil stresses (Dias *et al.* 2002; Dias 2003; Fita *et al.* 2005).

Some accessions of *C. melo* were characterized against the potyvirus (Potyviridae) melon yellows virus (MYV) using whitefly-mediated inoculation with the transmission vector *Trialeurodes vaporariorum* (Nuez *et al.* 1999).

Part of the collection of *C. sativus* landraces has been tested against the ipomovirus (Potyviridae) cucumber vein yellowing virus (CVYV), recently introduced into Spain. The assays are performed using sap inoculation and the virus titre is analyzed in each genotype by molecular hybridization and real-time PCR (Picó *et al.* 2003, 2005).

Response of about 100 accessions of *C. pepo* and *C. maxima* to zucchini yellow mosaic virus (ZYMV) under natural infection conditions has also been assayed. Part of this collection has been characterized molecularly (Ferriol *et al.* 2003a, 2003b, 2004).

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Scientific contributions

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Breeding melons for resistance to melon vine decline – the role of cucurbit germplasm

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Introduction

Melon (*Cucumis melo* L.) is cultivated all over the world. The main producers are China, the USA and Turkey. After the tomato, it is the most widespread vegetable crop in Spain with regard to surface area and production, with 38 100 ha and 1 102 400 t in 2004 (FAOSTAT 2005). Spain is the fifth most important melon producer worldwide and the leader in Europe. Melon production in Spain is mostly concentrated in the areas of Castilla la Mancha, Andalusia, Murcia, Extremadura and the Valencian Community.

Cultivars belonging to the 'Piel de Sapo' or 'Amarillo' types (*Cucumis melo* var. *inodorus*), grown under open-air or greenhouse conditions, are the most popular in the Spanish market. Other cultivars of the cantaloupe type (*Cucumis melo* var. *cantalupensis*) are mainly grown as early crops in greenhouses as their production is mainly oriented toward European markets.

The major pests of melon crop in Spain are the two-spotted spider mite (*Tetranychus urticae* Koch), the western flower thrips (*Frankliniella occidentalis* Pergande), various whiteflies (*Bemisia tabaci* Genadius and *Trialeurodes vaporariorum* West), aphids (*Myzus persicae* Sulzer and *Aphis gossypii* Glover) and leafminers (*Liriomyza* spp). These are very harmful not only as pests, but also as vectors for various viral diseases. The most significant viruses in the open field are those transmitted by aphids such as cucumber mosaic virus (CMV), watermelon mosaic virus (WMV-2), zucchini yellowing mosaic virus (ZYMV) and papaya ringspot virus (PRSV). In glasshouse-grown melon, the most significant viruses are melon necrotic spot virus (MNSV), which is transmitted by the soil-borne fungus *Olpidium bornovanus*, and cucumber vein yellowing virus (CVYV) and cucumber yellow stunting disorder virus (CYSDV), which are both transmitted by *B. tabaci*.

Besides viral diseases, there are widespread fungal diseases affecting melon in Spain such as powdery mildew (*Sphaerotheca fuliginea* syn. *S. fusca*), soil-borne fungal diseases such as fusarium wilt, which is caused by *Fusarium oxysporum* f.sp. *melonis*, and melon vine decline, also referred to as melon dieback, collapse or sudden wilt, which causes plant death. This syndrome, which has been associated with various pathogens, causes the greatest losses in melon cultivation in Spain.

Melon vine decline: economic importance and causal agents

Vine decline is a major root-rot disease in melon, not only in Spain but also in hot arid and semi-arid regions of the world (Martin and Miller 1996; Cohen *et al.* 2000). Although no reliable data exist about the actual incidence of melon vine decline in the Spanish Mediterranean littoral, it is known that the surface area used for melon cultivation has decreased in the last 20 years due to this disease (García Jimenez *et al.* 1989; Martyn and Miller 1996). The reported average yield in Castellón, one of the most badly affected areas in eastern Spain, is around 1.6 kg/m², which is low in comparison with the 3.4 kg/m² yield in Ciudad Real (a non-infested region in central Spain) (MAPA 2004).

Several soil-borne fungi, viruses and even bacteria have been related to the causation of this complex disease. In eastern Spain, *Acremonium cucurbitacearum* Alfaro-García, W. Gams et García-Jimenez and *Monosporascus cannonballus* Pollack et Uecker have been reported as the main causal agents (Martyn and Miller 1996). Of these two fungi, *M. cannonballus* seems

to be the most aggressive, so much so that the latest papers refer to this disease as *Monosporascus* sudden wilt or *Monosporascus* collapse (Pivonia *et al.* 2002a; Stanghellini *et al.* 2004a; Cohen *et al.* 2005). This disease is characterized by a sudden and generally uniform canopy collapse of the entire field 1-2 weeks prior to harvest, resulting in fruit sunburn and total crop loss. These foliar symptoms result from damage to the root system, which starts to occur much earlier. The fungus begins to invade the root system from as early as 2 weeks after planting, depending on soil temperature, causing root lesions, root rot and the loss of smaller feeder roots.

Control strategies: importance of cucurbit germplasm in breeding melons against vine decline

Various approaches have been tried out in order to control vine decline. Pre-planting fumigation of pathogen-infested fields with methyl bromide proved efficient for disease control, but its prohibition has spurred the need for alternative management strategies (Cohen *et al.* 2000). Other pre-planting soil fumigants like iodomethane and chloropicrin have been shown to be effective in reducing root-rot severity caused by *M. cannonballus* (Stanghellini *et al.* 2003). However, the persistence of the inoculum makes the use of complementary actions to avoid the disease necessary. Because *M. cannonballus* is a heat-tolerant fungus, traditional soil solarization is ineffective at controlling it. However, some improved solarization methods, or solarization in combination with other fumigants, might be useful in the control of the disease (Pivonia *et al.* 2002b). Biological control using hypovirulent phenotypes is still under study (Batten *et al.* 2000). Some cultural practices such as drip irrigation enhance the tolerance of the plants against wilt. The response of melon plants to vine decline may be attributed in part to the size and structure of the root system, which can be manipulated by the irrigation regime (Cohen *et al.* 2000). However, the efficacy of some agricultural practices considered effective in controlling the disease needs to be further studied. For example, Stanghellini *et al.* (2004b) recently demonstrated that crop residue destruction strategies enhance rather than inhibit the reproduction of *M. cannonballus*.

The use of resistant cultivars is one of the best alternatives for reducing damage caused by plant diseases. Wild relatives of *Cucumis melo* provide a wide range of variation for resistance to several diseases (Robinson and Decker-Walters 1997), but this variation is not easy to use since sexual crosses among *C. melo* and wild *Cucumis* species do not produce fertile hybrids (Chen and Adelberg 2000). Fortunately, genetic variability within the species *C. melo* is broad enough to find some tolerant cultivars. In the USA, tolerance to collapse syndrome has been reported in the cultivar 'Doublon', a 'Charentais'-type also resistant to MNSV (Wolff 1996; Wolff and Miller 1998; Crosby 2000; Crosby *et al.* 2000). In Israel, the resistance found in the cultivar 'Black Skin' from Taiwan is being introduced into 'Galia'-type melons (Cohen *et al.* 2000). In Spain, our group selected the accession *Cucumis melo* subsp. *agrestis* Pat 81 because of its resistance under field and artificial inoculation conditions (Esteve and Nuez 1994; Iglesias and Nuez 1998; Dias *et al.* 2004). Due to the high level of resistance found in Pat 81, this accession was selected to initiate a breeding programme aimed at introgressing its resistance into the most important Spanish melon types, 'Piel de Sapo' and 'Amarillo Canario' (Iglesias *et al.* 2000; Dias 2003). This programme has reached the third and fourth backcross generations, and the first resistant pre-commercial materials are being tested now.

Another possibility for controlling vine decline is the use of *Cucurbita* germplasm as rootstocks for melon (Cohen *et al.* 2002). The *Cucurbita* genus was reported by Mertely *et al.* (1991) as a host for *M. cannonballus*. However, the slow development of the pathogen in *Cucurbita* roots and their large root systems enable the melon-grafted plants to complete the growing season. In the past, grafted cucurbits were not used in Spain because of the

availability of methyl bromide for soil disinfection, but this situation is rapidly changing. Currently, grafted watermelons are grown commercially in Spain to control fusarium wilt. The cultivation of grafted melons to control fusarium wilt and melon vine decline is still under study. Garcia-Jimenez *et al.* (1990) tested 18 cucurbit rootstocks against *A. cucurbitacearum*, and found that 11 were resistant to the pathogen. However, neither the rootstock/scion compatibility nor the performance of the grafted plants in the field was assessed. Other studies report variable results from grafting melons onto *Cucurbita* rootstocks. In general, the use of grafted plants reduces the wilt incidence, but the response in terms of yield or quality depends on many factors (Edelstein *et al.* 1999; Traka-Mavrona *et al.* 2000; Cohen *et al.* 2005). It might be possible to reduce problems with rootstock/scion compatibility and detrimental effects on fruit quality by using a melon rootstock instead of *Cucurbita* (Nisini *et al.* 2002). The use as rootstock of the *Monosporascus*-resistant accession Pat 81, belonging to the subspecies *agrestis* of *C. melo*, is currently being researched at the Institute for Conservation and Improvement of Valencian Agrodiversity (Centro de Conservación y Mejora de la Agrodiversidad Valenciana, COMAV). These studies indicate that grafting can be an effective method for managing melon *Monosporascus* vine decline. However, since the rootstocks are also infested with *M. cannonballus*, the potential for inoculum build-up in the soil by continuous use of these rootstocks should be taken into consideration. The repeated use of grafted plants should be accompanied by other management strategies to avoid the risk of reaching an inoculum level high enough to overcome the partial resistance of the rootstock to the disease.

Conclusions

There are several strategies for controlling vine decline. However, none of them are 100% efficient. Future strategies for controlling this soil-borne disease will probably be based on an integrated management approach, combining improved use of the available germplasm (by grafting or by the introgression of resistance genes) with various techniques for reducing the inoculum level in the soil.

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Assessing procedures for optimal regeneration of *Cucurbita* spp. at COMAV

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Introduction

Gene banking is a way of maintaining genetic diversity and making it available to plant breeders and other researchers. The Genebank at the Institute for Conservation and Improvement of Valencian Agrodiversity (Centro de Conservación y Mejora de la Agrodiversidad Valenciana, COMAV) at the Polytechnic University of Valencia maintains a collection of more than 7000 accessions belonging mainly to the 17 most important species cultivated as vegetables in Spain (mostly Brassicaceae, Solanaceae and Cucurbitaceae) (Díez *et al.* 2002). The collection of cucurbit genetic resources is one of the largest ones at the Institute (Picó *et al.* 2002). Most of the accessions belong to cultivated species of three genera: *Cucumis*, *Citrullus* and *Cucurbita*. The COMAV maintains one of the world's largest collections of *Cucurbita* spp., with more than 1000 accessions.

The cultivated species of *Cucurbita* spp. were domesticated in different areas of America (Sanjur *et al.* 2003). Spain acted as a bridge between Latin America and Europe for the introduction and exchange of these species. Their historical evolution in our country has produced a large number of landraces with unique characteristics in different regions. The COMAV collection includes landraces of the species *C. pepo*, *C. maxima*, *C. moschata* and *C. ficifolia* from all the regions of Spain as well as from several Latin American countries. This collection is undoubtedly a valuable reservoir of interesting genes for *Cucurbita* breeding (Ferriol *et al.* 2003, 2004a, 2004b).

Efficient *ex situ* conservation relies on maintaining the genetic integrity of the original accessions. The regeneration procedure is an essential part of any conservation programme. Genetic diversity losses are associated with both the frequency of regeneration and the regeneration methods. The required frequency of regeneration is a function of initial sample size, of usage demand, and of the length of seed viability under the storage conditions. Recent improvements in seed storage techniques allow longer conservation, thus reducing the frequency of regeneration (Breese 1989).

However, in each regeneration cycle, the genetic integrity of each accession is threatened by contamination (alien pollen, seed mixtures) and genetic erosion (genetic drift, genetic shift due to unconscious selection). Contamination is strongly dependent on the breeding system, which determines the risk of outcrossing. *Cucurbita* species are assumed to be entomophilous and open-pollinated (Whitaker and Davis 1972) and the use of insect-proof cages is particularly useful in avoiding alien pollen contamination. Some institutions use isolation cages and insect-pollination (by honey bees or bumble bees) to regenerate *Cucurbita* accessions. However, cages are quite expensive (especially the large ones required for *Cucurbita* species) and insect management is tedious and costly. Therefore, in many institutions, the multiplication of *Cucurbita* species is performed by flower bagging and hand-pollination under greenhouse or open-air conditions (Díez *et al.* 2002). Various different hand-pollination methods are commonly used, and the regenerated samples usually consist of seed mixtures from self- or cross-pollinated fruits.

Appropriate regeneration methods also require the use of populations large enough to preserve segregating alleles within the accession samples. For example, optimal population sizes proposed for different cucurbits are from 15 to 22, and they need to be from 30 to 45 to maintain alleles with frequencies of 10% and 5%, respectively (Esquinas Alcázar and Güllick

1983). However, large collections have many problems in attempting to follow these standards.

We have initiated a series of studies aimed at establishing guidelines for regeneration and multiplication of species of *Cucurbita*. In order to do so, we are performing trials to compare different population sizes, different pollination techniques and different methods of seed management. Genetic diversity is being estimated using molecular markers. The strategies and tactics that are optimal to limit genetic drift and preserve an acceptable level of genetic diversity within the collection will be selected.

Materials and methods

Plant material

We chose to work on 43 accessions of *C. pepo*, 25 accessions of *C. moschata*, 10 accessions of *C. maxima* and 10 accessions of *C. ficifolia*, which represent the variability of origin, subspecies, and morphotypes held in the COMAV collection. Twenty-seven accessions of *C. pepo* were cultivated under open-air conditions together with the accessions of the other species. The remaining accessions of *C. pepo* were cultivated in a greenhouse. In a preliminary experiment, 5 plants per accession were grown. Further experiments are being conducted with 20 plants per accession and a smaller number of accessions.

Pollination methods

Each plant was self-pollinated (selfings, S), and also used as a female or male parent in a cross with another plant of the same accession (controlled cross-pollination, CCP). During this crossing process, female and male flowers were bagged before opening to avoid contamination. Fruits which set naturally (probably by insect-pollination) were allowed to mature (uncontrolled pollination, UC). All the fruits set were collected when mature, and the number of seeds per fruit was counted. Germination percentages of seeds obtained with different pollination methods were determined in certain selected accessions using 3 samples of 100 seeds each.

Molecular analysis

Two of the accessions trialled (one of *C. maxima* and the other of *C. moschata*) were selected for a preliminary molecular assay. The genetic diversity of the seed samples obtained using the different pollination methods was assessed and compared with that of the original sample. Bulks of DNA from 5 different plants were analyzed using amplified fragment length polymorphism (AFLP) markers following the procedure reported by Ferriol *et al.* (2003, 2004a, 2004b).

Statistical analysis

The data obtained from the fruit evaluations were analyzed using analysis of variance (Statgraphics plus). Molecular data were analyzed by cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) and the Dice coefficient (Nei and Li 1979) (NTSYSpc).

Results and discussion

Pollination efficiency, number of seeds, and germination rates

The different species displayed different behaviours with regard to the percentage of fruits set using different pollination methods, the number of seeds per fruit and the germination rate of seeds.

For all the species, a high percentage of the fruits (25-76%) came from uncontrolled pollinations (UP), probably caused by insects (Table 1). Insect-pollination is one of the main sources of contamination when isolation cages are not used. This problem is more significant in fields than in greenhouses, as the number of pollinators inside greenhouses is usually low. In fact, despite not taking special measures to eliminate pollinators, the percentage of UP fruits of *C. pepo* accessions that we cultivated in the greenhouse dropped to 2.3%. Therefore, greenhouses, when available, can help to reduce contamination during the regeneration of *Cucurbita* species.

Table 1. Percentage of fruits obtained from different *Cucurbita* species using different pollination methods

Species	Growing conditions	Selfing	Controlled cross-pollination	Uncontrolled pollination
<i>C. pepo</i>	Open-air	30.2	19.1	50.7
	Greenhouse	72.7	25.0	2.3
<i>C. moschata</i>	Open-air	33.9	22.9	43.2
<i>C. maxima</i>	Open-air	46.7	28.3	25.0
<i>C. ficifolia</i>	Open-air	7.2	16.4	76.4

Hand-pollination was successful in producing fruits, both from self- and from cross-pollination. However, several differences were found between the species (Table 1). The efficiency of hand-pollination was very low for *C. ficifolia* in comparison with natural pollination (24% compared to 76%). This result could be due to the poor adaptation of this species to our mild climate. *C. ficifolia* has a strong preference for cool, high-elevation ecological zones and flowered with difficulty in our experimental conditions. Flowering problems negatively affected hand-pollinations, as sometimes it was difficult to find simultaneously both female and male flowers in one plant/accession. Uncontrolled pollinations were less affected, since insects can get pollen from any available flower, that is flowers from the same accession that were not available at the moment of hand-pollination and flowers from other accessions.

The open-pollinated nature of the species of *Cucurbita* led us to think that selfing might be less efficient than cross-pollination. However, we did not find significant differences in the efficiency of fruit set with both pollination systems (Table 1). The percentage of fruits that came from self-pollinations was higher because we performed more self-pollinations than cross-pollinations. Therefore self-pollination can be used in the regeneration of *Cucurbita* species (Table 1). In any case, we have to consider that fruit set percentages can be skewed due to the effect of fruit load. The size of *Cucurbita* fruits makes some accessions unable to support more than 3 or 4 fruits per plant. This self-limitation in fruit number could be the cause of the low percentage of fruits coming from uncontrolled pollinations in *C. maxima*.

The number of seeds per fruit was counted (Table 2). No significant differences were found between self- and cross-pollination for most of the species in the trials. Only for *C. moschata* were significant differences found, and in this case, self-pollinated fruits had more seeds than those resulting from cross-pollination. In general, fruits coming from uncontrolled pollinations had fewer seeds. The number of seeds found in these fruits was much more variable than the number found in fruits coming from hand-pollination. These results reinforce the utility of hand-pollination methods for *Cucurbita* regeneration, particularly in terms of number of seeds obtained per fruit. These results also suggest that inbreeding depression does not significantly affect the number of seeds, at least in the first cycle of self-pollination, for most of the species. It is worth mentioning the different behaviour of *C. maxima*. In this species the number of seeds found in fruits coming from

uncontrolled pollinations is significantly higher than that found in fruits that come from hand-pollinations. This could be due to inbreeding depression. It has been reported that inbreeding depression mainly acts in the first developmental stages of the embryo and results in non-viable seeds. Only the seeds which were apparently viable were counted in each fruit. The lack of differences between self- and cross-pollinations could be a consequence of low intra-accession variability. This situation should be taken into account during the regeneration of this species.

Table 2. Analysis of variance (Anova) results for the number of seeds per fruit obtained from different *Cucurbita* species using different pollination methods

	<i>C. pepo</i>		<i>C. moschata</i>	<i>C. maxima</i>	<i>C. ficifolia</i>
	Open-air	Greenhouse	Open-air	Open-air	Open-air
Selfing	311.2 a	137.9 a	391.9 a	102.4 a	261.9 a
Controlled cross-pollination	259.6 ab	164.9 a	331.5 b	109.6 a	250.4 a
Uncontrolled pollination	226.5 b	-	244.7 c	235.8 b	210.3 a
<i>P</i> -value	0.0200	0.7345	0.0000	0.0000	0.5670

Each number is the mean of all the fruits of each species obtained with each pollination procedure.

Numbers in the same column followed by the same letter are not significantly different according to the Duncan test for mean comparisons.

We found variability within species in the response of the different accessions (Fig. 1). The level of intra-specific variation was different in the species assessed. Accessions of *C. moschata* and *C. maxima* behaved more uniformly than accessions of *C. pepo*. This result is not surprising as we included accessions belonging to the two subspecies of *C. pepo*. These two independently domesticated subspecies display striking differences (Paris *et al.* 2003; Ferriol *et al.* 2003).

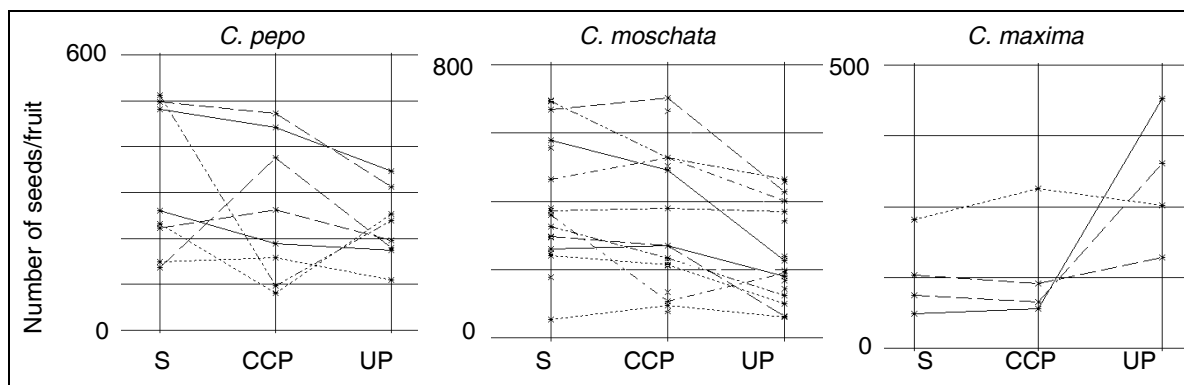


Fig. 1. Number of seeds per fruit obtained from different *Cucurbita* species using different pollination methods (S: selfing; CCP: controlled cross-pollination, crossing two plants of the same accession; UP: uncontrolled pollination). Each line represents one accession. Each point is the mean of all the fruits obtained from this accession with each pollination method. Only accessions with more than 3 fruits set per pollination method are included.

The preliminary germination test performed using 4 selected accessions showed that there were no significant differences in germination ratios among the seed lots obtained with different pollination methods (the mean germination rate was 84.1%, 85.1 % and 79.29% for S, CCP and UP, respectively) (Fig. 2). This result is not surprising as we only extracted from each fruits those seeds that were apparently viable and thus not affected by severe embryo alterations due to inbreeding depression. We should also note that only the final germination

rate was studied, and further analysis is required in order to study the likely effect of inbreeding depression in delaying germination.

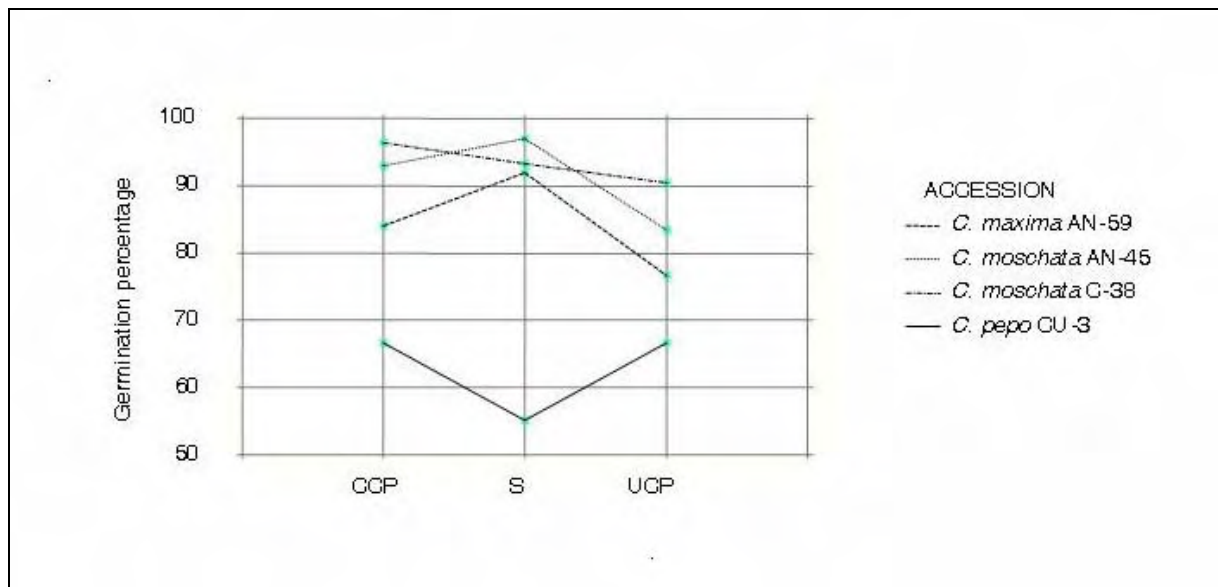


Fig. 2. Germination percentage of seeds obtained from different *Cucurbita* species using different pollination methods (S: Selfing; CCP: controlled cross-pollination, crossing two plants of the same accession; UP: uncontrolled pollination). Each line represents one accession. Each point is the mean of 3100 seed samples obtained from this accession with each pollination method.

In conclusion, our preliminary results show the efficiency of hand-pollination methods in regenerating *Cucurbita* accessions as an alternative to insect-pollination using isolation cages. In terms of fruit set, number of seeds per fruit, and germination rates, these methods are successful for *Cucurbita* regeneration. However, hand-pollination is labour-intensive, especially for selfing methods in which each plant has to be selfed to avoid the loss of genetic diversity. We are currently comparing other methods such as pollination with pollen mixtures which can reduce the number of pollinations, thus ensuring the preservation of genetic diversity.

Molecular analysis

AFLP markers were used to determine the efficiency of the different pollination methods in preserving the genetic integrity of the original accession. In a preliminary survey, two accessions were selected for study. The results showed significant differences between the two accessions in the amount of genetic diversity of the original sample (Fig. 3). The three DNA bulks of the original sample of the *C. maxima* accession (C.ma-o) which were analyzed appear grouped in the cluster, whereas the two bulks of the original sample of *C. moschata* (C.mo-o) cluster separately. This is an important result. Due to the open-pollinated nature of the *Cucurbita* species, a high genetic variation is supposed to exist within a single cultivar. Thus the use of large populations is recommended during regeneration to preserve segregating alleles. However, this is not always true for cultivated *Cucurbita*, as cultivars are often grown in very small populations. For example, the Spanish landraces of *Cucurbita* are mainly grown under traditional agricultural systems, where a very few plants of each type and species are grown in small plots for domestic consumption or to be sold in local markets.

Our results illustrate this point, with one accession of *C. maxima* which had a very low intra-accession genetic diversity.

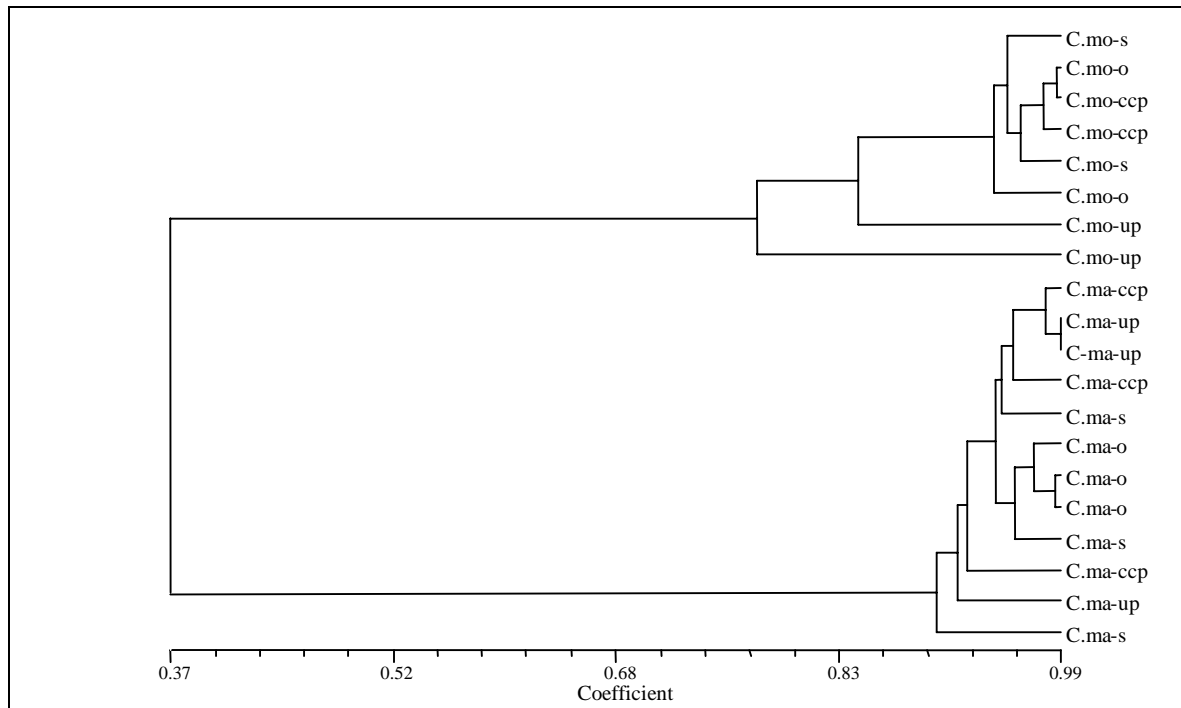


Fig. 3. Cluster analysis of different DNA samples analyzed with AFLPs of one accession of *C. moschata* and *C. maxima* obtained using different pollination procedures (S: Selfing; CCP: controlled cross-pollination, crossing two plants of the same accession; UP: uncontrolled pollination) in comparison with the original sample (o). UPGMA and the Dice coefficients were used.

In the two accessions assessed, both the mixture of seeds coming from self-pollinated fruits (s) and the mixture of seeds coming from cross-pollinated fruits (ccp) presented the variability found in the original samples (o). However, the two accessions behaved differently with regard to the bulks coming from uncontrolled pollinations (Fig. 3). For *C. moschata*, these bulks (C.mo-up) cluster separately from the other bulks (C.mo-s, C.mo-ccp, C.mo-o), which means that a contamination of alien pollen (from other accessions of *C. moschata*) probably occurred. However, the situation for *C. maxima* is quite different. Here, the bulks from uncontrolled pollinations (C.ma-up) grouped together with bulks coming from self- and cross-pollinations. This could indicate that uncontrolled pollinations come from crosses occurred among plants of the same accession or with plants of another accession but which were genetically similar.

Although preliminary, the results of the molecular analysis provide information that may be very useful for determining optimal strategies on a species by species basis, based on genetic as well as economic considerations. Analyses of individual plants and with larger numbers of accessions are being conducted to further compare the different hand-pollination methods, including the use of pollen mixtures.

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Appendix I. Useful Web addresses for cucurbit genetic resources and cucurbit taxonomy¹⁷

- **Mansfeld's World Database of Agricultural and Horticultural Crops**
<http://mansfeld.ipk-gatersleben.de/mansfeld/Query.htm>
- **Germplasm Resources Information Network (USDA)**
<http://www.ars-grin.gov/npgs/>
- **Cucurbit Genetics Cooperative**
<http://cuke.hort.ncsu.edu/cgc/>
- **World Information and Early Warning System on PGRFA (WIEWS)**
<http://apps3.fao.org/wiews/>
- **Cucurbitaceae**
<http://www.cucurbit.org/family.html>
- **The International Plant Names Index (IPNI)**
<http://www.ipni.org/ipni/plantnamesearchpage.do>
- **Kokopelli Seed Foundation**
<http://www.kokopelli-seed-foundation.com>
- **Searchable World Wide Web Multilingual Multiscript Plant Name Database**
<http://www.plantnames.unimelb.edu.au/Sorting/Frontpage.html>

¹⁷ At time of publication, this list is available on-line and regularly updated (http://www.ecpgr.cgiar.org/Workgroups/Cucurbits/U_links.htm)

Appendix II. General guidelines for regeneration of cucurbit species

This protocol gives general guidelines for a successful regeneration. Conditions and available equipment can vary.

Disinfection of seeds

- Examples of treatments which can be used (depending on the crop, other treatments are possible)
 - **Cucumber:** before sowing, seeds may be disinfected. Disinfection can be done by maintaining seeds at a temperature of +76°C for 72 hours using dry heated air. Before the heat treatment seeds must be dried to a moisture content not higher than 6% (seeds with a higher moisture content may be damaged during the heat treatment).
 - **Cucurbita:** seeds can be disinfected with sodium hypochlorite, 150 ml/l commercial bleach, for 5 minutes.

Identification

- Plants must be properly labelled with a unique number during regeneration to prevent any mix-up of accessions. From sowing until harvest the same number will be used for one accession.

Number of plants regenerated

- At least 10-20 plants per accession should be used but more is better. For heterogeneous accessions at least 15 plants must be used to ensure the preservation of genetic diversity.

Transplanting seedlings or elimination of seedlings in field plots

- From the seedlings, the needed number of plants for regeneration must be picked without making any selection. Only seedlings that are not fit enough to grow and reproduce can be skipped or removed.

Isolation

- To prevent outcrossing each accession must be isolated. This can be done by putting accessions in an insect-free glasshouse, or by isolating accessions with gauze nets, or planting in isolation fields, or by bagging the flowers.
- For seed production several alternative methods can be used:
 - to collect and mix pollen from all plants of one accession and use it to pollinate all plants. This method does not prevent selfing completely, but the probability of selfing is low.
 - the so-called chain pollination, carried out by hand. Per accession plant 1 is crossed with plant 2, plant 2 with plant 3, etc.
 - to isolate accessions in isolation cages to be pollinated by insects such as bumble bees.

Treatments during regeneration

- If the plant gives predominantly male flowers it is possible to obtain female flowers by pruning. If pruning does not help, the plants can be sprayed with ethylene. Plants with only female flowers can be induced to produce male flowers after treatment of the sprouts with 250-ppm silver nitrate.

- Only remove a plant during regeneration if it is very clear that it is an off-type because a mix-up has taken place.

Harvest

- To be sure that each plant of the accession contributes equally to the seed lot, on average an equal number of comparable fruits per plant must be harvested. Do not make selections during fruit harvesting.

Processing

- Seeds should be dried as quickly as possible but not in full sunshine or at very high temperatures because this affects the germination capacity of the seeds.
- After first drying the seeds can be packed in paper bags for further drying with silica gel or in an air-conditioned room with controlled air humidity and temperature.
- Well dried seeds must be stored airtight under cool conditions. Frozen seeds (first dried down to a safe moisture content) will keep their germinability longer.

Appendix III. Workplan of the Working Group on Cucurbits

Agreed at the First meeting of the ECP/GR Working Group on Cucurbits, 1-2 September 2005, Plovdiv, Bulgaria

Action	Carried out by	Date by when action should be completed
Prepare a proposal for a Cucurbits project to be presented to the next EU Genetic Resources Programme	Chair and Vice-Chair	Ongoing
<u>Improve the ECCUDB</u>		
Modify the structure of the ECCUDB for the inclusion of primary characterization data	Alvaro Gil (Database manager) and M.J. Díez	Four months after the first meeting of the CWG (by January 2006)
Send missing passport data to the DB manager	WG members of countries not yet included in the DB	March 2006
Enter passport data into the DB	DB manager	March 2006
Keep the database updated with new passport data	All and DB manager	Ongoing
Add to the database a taxonomy information module	DB manager and M.J. Díez	January 2006
Send available characterization data to DB manager (see minimum descriptors agreed by the Group, http://www.ecpgr.cgiar.org/Workgroups/Cucurbits/Cucurbits.htm)	All WG members	When/if possible
Send new characterization data to DB manager (see minimum descriptors agreed by the Group, http://www.ecpgr.cgiar.org/Workgroups/Cucurbits/Cucurbits.htm)	All WG members	As soon as accessions are regenerated and characterized
Link the WG Web page to cucurbit information pages (see Appendix I)	ECP/GR Secretariat	November 2005
Circulate the draft guidelines for regeneration and the draft minimum descriptor lists for characterization for approval by the WG members	Chair	October 2005
Seek IPGRI's comments on the above minimum descriptor lists	Vice-Chair	As soon as descriptors are approved by the WG
Make available on the Web page the guidelines for regeneration of all cucurbit crops (see Appendix II)	ECP/GR Secretariat	One month after approval of the guidelines by the WG
Make available on the Web page the minimum descriptor lists for characterization of melon, cucumber, watermelon and <i>Cucurbita</i> spp.	ECP/GR Secretariat	One month after approval of the descriptors by the WG
Identify experts on minor cucurbits in order to develop the respective minimum descriptor lists	L. Horváth	Before the next meeting of the WG
Complete the draft table of safety-duplication status with information from members who did not attend the Plovdiv meeting	Vice-Chair	December 2005
Promote safety-duplication of each collection under long-term conservation conditions	All Working Group members	Ongoing

Appendix IV. Acronyms and abbreviations

AARI	Aegean Agricultural Research Institute, Izmir, Turkey
ABI	Institute for Agrobotany, Tápiószele, Hungary (<i>now Research Centre for Agrobotany, Central Agricultural Office</i>)
AEGIS	A European Genebank Integrated System
AFLP	Amplified fragment length polymorphism
BPGV	Banco Português de Germoplasma Vegetal, Braga, Portugal
CGN	Centre for Genetic Resources, Wageningen, The Netherlands
CIDA	Centro de Investigación y Desarrollo Agroalimentario, Spain
CIFA	Centro de Investigación y Formación Agraria, Spain
CMV	Cucumber mosaic virus
COMAV	Centro de Conservación y Mejora de la Agrodiversidad Valenciana (Institute for Conservation and Improvement of Valencian Agrodiversity), Polytechnic University of Valencia, Spain
CRF	Centre for Genetic Resources, Madrid, Spain
CRI	Crop Research Institute, Prague-Ruzyne, Czech Republic
CSIC	Consejo Superior de Investigaciones Científicas, Spain
CVYV	Cucumber vein yellowing virus
CYSDV	Cucumber yellow stunting disorder virus
DGPA	Dirección General de la Producción Agraria, Castilla-La Mancha, Spain
EAN	Estação Agronómica Nacional, Oeiras, Portugal
ECCUDB	European Central Cucurbits Database
ECP/GR	European Cooperative Programme for Crop Genetic Resources Networks (<i>now ECPGR</i>)
ECPGR	European Cooperative Programme for Plant Genetic Resources
ENMP	Estação Nacional de Melhoramento de Plantas, Portugal
EPGRIS	European Plant Genetic Resources Information Infra-Structure
EC	European Commission
ENEA	Ente per le Nuove Tecnologie, l'Energia e l'Ambiente (Italian National Agency for New Technologies, Energy and the Environment), Roma, Italy
EPSO	Escuela Politécnica Superior de Alicante, Spain
EU	European Union
EURISCO	European Internet Search Catalogue (EPGRIS project)
FAO	Food and Agriculture Organization of the United Nations, Rome, Italy
IAS	Instituto de Agricultura Sostenible, Córdoba, Spain
IGV	Istituto di Genetica Vegetale, Bari, Italy
INRA	Institut National de la Recherche Agronomique (National Institute for Agronomical Research), France
IPGR	Institute for Plant Genetic Resources, Sadovo, Bulgaria
IPGRI	International Plant Genetic Resources Institute, Rome, Italy (<i>now Bioversity International</i>)
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung (Institute of Plant Genetics and Crop Plant Research) (<i>now Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung</i>)
IVIA	Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain
MCPD	Multi-crop Passport Descriptors (FAO/IPGRI)
MNSV	Melon necrotic spot virus
MYV	Melon yellows virus

NCG	Network Coordinating Group (of ECPGR)
PGR	Plant genetic resources
PRSV	Papaya ringspot virus
RAPD	Random amplified polymorphic DNA
RICP	Research Institute of Crop Production, Prague–Ruzyne, Czech Republic (<i>now Crop Research Institute, CRI</i>)
RIVC	Research Institute for Vegetable Crops, Skierniewice, Poland
SBAP	Sequence-based amplified polymorphism
SIA	Servicio de Investigación Agroalimentaria, Spain
SIDT	Servicio de Investigación y Desarrollo Tecnológico, Spain
UPGMA	Unweighted pair group method with arithmetic mean
UPOV	Union internationale pour la Protection des Obtentions Végétales (International Union for the Protection of New Varieties of Plants), Geneva, Switzerland
UPV	Universidad Politécnica de Valencia (Polytechnic University of Valencia), Spain
USDA	United States Department of Agriculture
UTAD	Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal
VIR	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russian Federation
WMV-2	Watermelon mosaic virus
ZYMV	Zucchini yellow mosaic virus

Appendix V. Agenda

First Meeting of the ECPGR Working Group on Cucurbits 1-2 September 2005, Plovdiv, Bulgaria

Wednesday 31 August 2005

Arrival of participants

Thursday 1 September 2005

8:30 – 9:45

Introduction

- Introductory welcome from IPGR Sadovo (*L. Krasteva*) – 5 min
- Opening remarks (*L. Maggioni and M.J. Díez*) – 10 min
- Self-introductions by the participants – 1 min per person (15 min)
- Presentation of the agenda and adjustments – 5 min
- Selection of Chair for the meeting – 5 min
- Briefing on ECP/GR Phase VII and other PGR international events (*L. Maggioni*) – 20 min
- Acting Chairperson's report and outline of Cucurbits WG activities (*M.J. Díez and W. van Dooijeweert*) – 15 min

9:45-10:30

Reports on status of National Collections

Reports from countries not covered by the Adana report (2002): Collecting, conservation, safety-duplication, characterization or evaluation, regeneration, availability of material, institutional responsibilities, etc. (10 min. presentations from Italy, Poland, Serbia and Montenegro, Slovenia, etc.)

10:30–11:00

Coffee break

11:00-11:30

Reports on status of National Collections

Short update on National Collections – conservation, collecting, evaluation or characterization (5 min. presentations from Bulgaria, Czech Republic, Hungary, The Netherlands, Portugal, Spain, Turkey)

11:30–12:30

Mode of operation: Discussion of the workplan of the Cucurbits Working Group and its schedule

- Introduction (*M.J. Díez*) – 5 min
- Current state of the European Central Cucurbits Database (ECCUDB) (Presentation of the ECCUDB and its relation with EURISCO) (*introduced by M.J. Díez*) – 10 min
- Updating passport data at CGN (*W. van Dooijeweert*) – 10 min
- Planning for safety-duplication of each collection under long-term conservation conditions (*introduced by W. van Dooijeweert*) – 20 min

12:30–14:00

Lunch

- 14:00–15:30 Mode of operation (cont.)**
- The need for minimum descriptor lists (5 min)
 - Establishment of minimum descriptor lists for each crop (55 min)
 - *Cucumis melo*
 - *Cucumis sativus*
 - *Citrullus lanatus*
 - *Cucurbita* spp.
 - Establishment of regeneration protocol and storage for cucurbits (30 min)
- 15:30–16:00 Coffee break**
- 16:00–17:30 Information on identification of cucurbit material on the Internet**
(Introduced by M.J. Díez)
Discussion on the most relevant problems of cucurbit cultivation in each country
Short contributions (10 min.) are welcome
Other scientific and technical contributions
Short contributions (10 min.) are welcome

Friday 2 September 2005

- 8:30–12:30 Report drafting / Excursion**
 Drafting of the report. For those not involved in the drafting, a visit will be organized to the Bachkovo Monastery
- 12:30–14:00 Lunch**
- 14:00–15:30 The way ahead**
- Opportunities for funding the Working Group's activities (EC programmes)
 - Perspectives for the future of the Working Group on Cucurbits
- 15:30–16:00 Coffee break**
- 16:00–18:00 Conclusion**
- Presentation of the report and adoption of recommendations
 - Selection of the Working Group's Chair and Vice-Chair
 - Closing remarks
- Evening Social dinner*

Saturday 3 September 2005

Departure of participants

Appendix VI. List of participants

N.B. Contact details updated at time of publication. The composition of the Working Group is subject to changes, and is constantly updated on the Working Group's Web page (http://www.bioversityinternational.org/networks/ecpgr/contacts/ecpgr_wgcu.asp).

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