European Cooperative Programme for Crop Genetic Resources Networks

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European collections of vegetatively propagated *Allium*

IPGRI

Report of a Workshop, 21–22 May 2001, Gatersleben, Germany

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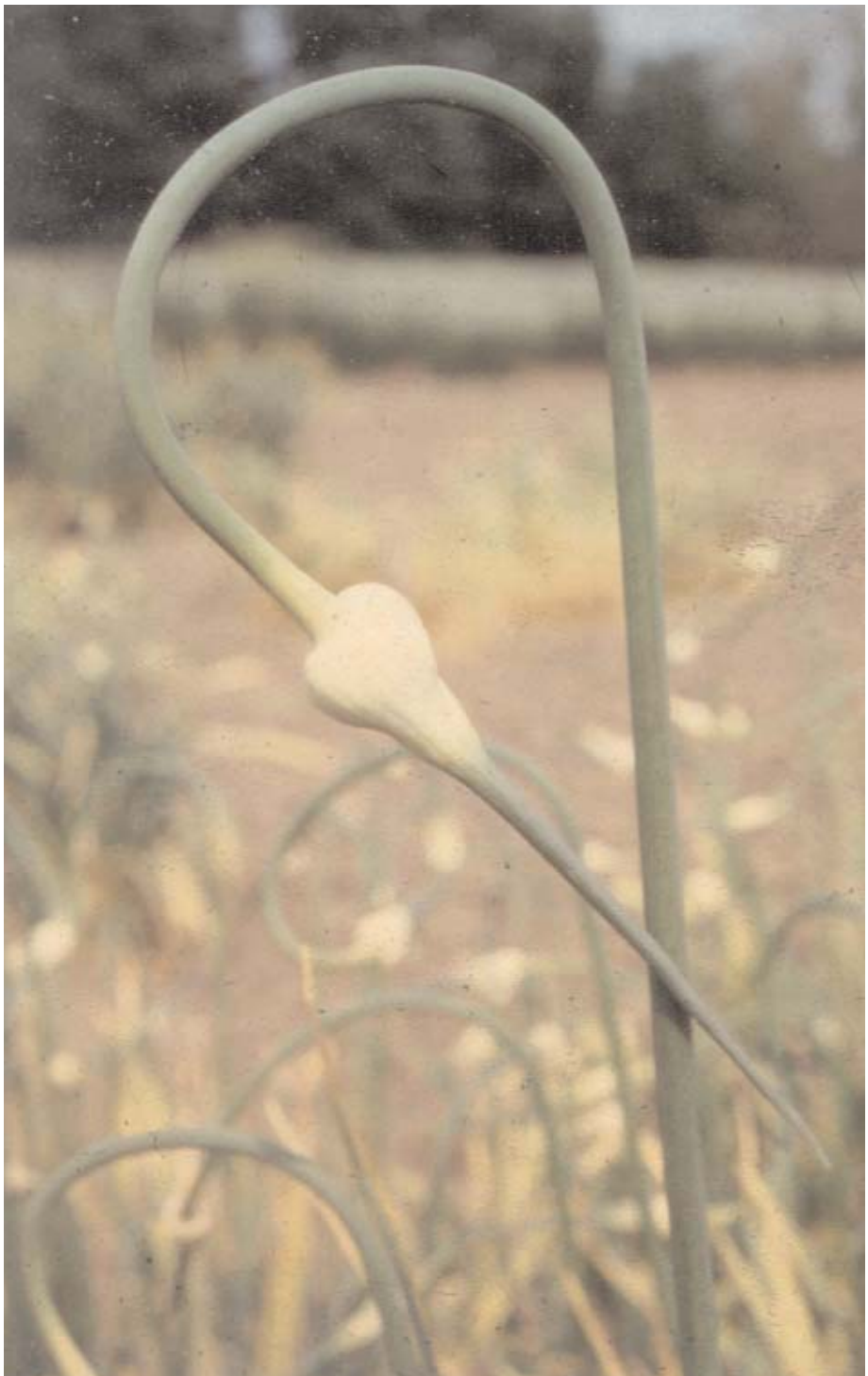

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The **International Plant Genetic Resources Institute (IPGRI)** is an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advance the conservation and use of genetic diversity for the well-being of present and future generations. IPGRI has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme and (3) the International Network for the Improvement of Banana and Plantain (INIBAP).

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Part I. Discussion and Recommendations

Introduction

The workshop was opened by Andreas Graner, Head of the Genebank Department of the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK-Gatersleben), who welcomed all the participants. He then thanked the ECP/GR Secretariat for convening the workshop and his collaborators, Joachim Keller and Angelika Senula, for their assistance in the local organization of the workshop. He also welcomed Peter Hanelt, former leader of the wild species collection, as a special guest and reminded everybody of P. Hanelt's past activity in the ECP/GR Working Group on *Allium*, including the fourth meeting held in Gatersleben in 1991.

He then went on to describe the IPK Institute, founded in 1943 and currently employing 146 scientists and a total of 423 staff. The Institute is spread over 70 hectares of land, of which between 15 ha and 20 ha are used by the Genebank. Major reconstruction activities are taking place, including the opening of the "Plant Genome Resources Centre", inaugurated in September 2000, directed by Patrick Schweizer. Since 1946 the Institute has been based in Gatersleben, where it was moved from its previous location in Vienna. Since its foundation, the major focus of the Institute has been directed towards plant science. At present, the three major research areas (plant genetic resources, genome research and molecular physiology) are organized in five scientific departments: Genebank, Taxonomy, Cytogenetics, Molecular genetics, and Molecular cell biology.

The genebank has two branch offices: potatoes and forage crops in Gross Lüsewitz and Malchow, and the fruit collection in Dresden-Pillnitz. The main tasks of the genebank are collection, conservation and documentation of plant genetic resources, research on cultivated plants and provision of plant material for research. The genebank is endowed with 65 permanent staff, plus 30 temporary research fellows.

The scientific teams of the genebank deal with molecular markers (A. Graner), documentation (H. Knüpffer), *in vitro* research (J. Keller), genetic resources reproduction at Gatersleben (A. Börner) and in the northern (K. Schüler) and southern branches (M. Fischer). The collection includes specimens from 600 genera and 2147 species, for a total of 102 325 accessions.

The *Allium ex situ* collection includes the main crop species: *A. sativum*, *A. cepa*, *A.* × *proliferum* and others, amounting to a total of 1465 accessions. In addition, the *Allium* taxonomy collection includes 1865 accessions from 275 taxa. Germplasm is maintained in cold room storage, in the field and *in vitro*. Collections are documented with passport, characterization and evaluation data. During the last year, 21 784 samples of the entire genebank have been dispatched throughout the world, free of charge.

Research at the genebank is focused on the identification of useful genetic traits to be deployed in breeding programmes for the improvement of crop species.

A. Graner concluded by wishing that the meeting could intensify the level of collaboration in Europe. He also confirmed that IPK was prepared to share responsibilities for the conservation of *Allium* and was committed to ensure the long-term maintenance of its collections.

J. Keller, Vice-Chair of the Working Group on *Allium* and local organizer of the meeting, expressed his personal wish that representatives from different countries use this event to prevent the loss of genetic resources and to find ways to counteract the recurrent shortage of funds. D. Astley, Chair of the Working Group on *Allium*, thanked the organizers and particularly J. Keller, also for his effort in the preparation of background documents (see Appendix I). He then expressed his appreciation for A. Graner's very positive comments regarding IPK's commitment for the maintenance of the collections. Finally, he reminded

the Group that the main concern related to the vegetatively propagated *Allium* collections was the high cost of conservation and the need to find solutions to ensure formal long-term commitments for the conservation of existing genetic diversity. He invited the participants to be informal and to develop proposals starting from a clear and frank discussion on how national representatives see the future of the collections.

The participants briefly introduced themselves and then presented the status of their respective collections.

Reports on the vegetatively propagated *Allium* sp. collections

(Full papers are included in Part II)

The European field collection of long-day Allium *species, Czech Republic*[1]

H. Stavělíková summarized the current state of the collection, which is based at the Olomouc Genebank, as part of the Czech national genebank. The collection was established following a decision made at the second meeting of the ECP/GR Working Group on *Allium* held in Olomouc in 1986. It currently consists of two parts, the garlic collection and the shallot collection.

The garlic collection includes 641 accessions originating from more than 15 countries. The shallot collection includes 119 accessions, mainly from Finland and Norway. All accessions are regularly regenerated every year. Part of the collection (25 garlic and 30 shallot accessions) is maintained in virus-free status in isolation cages. Computerized documentation of the collection is complete for passport data and includes a large proportion of characterization data (28 morphological descriptors for garlic, 13 for shallot).

Depending on the health of material and its availability in stock, 90% of the collection is available for distribution.

The loss of 16 shallot accessions during the flood of 1997 has stressed the need to implement safety-duplication measures and to introduce alternative methods of conservation. Part of the shallot collection is currently safety-duplicated at Gatersleben, Germany (20 accessions), Grimstad, Norway (17) and Skierniewice, Poland (15). Garlic is also safety-duplicated at Gatersleben, Germany (76 accessions).

Discussion

In reply to questions from the audience, H. Stavělíková added the following:

- The level of duplication within the collection was not tested with molecular markers. However, morphological characterization indicates the presence of some duplicates.
- Virus-free material is cleaned from OYDV (onion yellow dwarf potyvirus) through meristem tip cultures. Virus-free condition is maintained under sheet isolators and a symptomatic check is made every year to control possible virus re-infection. OYDV absence was verified by ELISA test in 1988. A new certification test with ELISA (four viruses) and PCR (two viruses) is planned for 2002.
- A dispatch of 100 accessions sent for safety-duplication from Bulgaria was found to have remained for several weeks in the post office in bad conditions and this has probably caused the loss of about 35 accessions.

[1] See also: Havránek, P. and H. Stavělíková. 1999. The European field collection of long-day *Allium* species. Pp. 31-34 *in* Report of a Working Group on *Allium*, Sixth meeting, 23-25 October 1997, Plovdiv, Bulgaria (L. Maggioni, D. Astley, H. Rabinowitch, J. Keller and E. Lipman, compilers). International Plant Genetic Resources Institute, Rome, Italy.

- Considering that the collection is now part of the Research Institute for Crop Production (RICP), Prague, it can be foreseen that the Czech government will continue to ensure maintenance of the material.
- Collaboration with the Slovak Republic for safety-duplication is ongoing. Material was sent to Piešťany in 1999. Olga Hornaková is the curator.
- In view of a possible further expansion of the collection, there would be no problem of space in the field, but storage and drying space are limited.

As a security measure to avoid losses in the field, T. Kotlińska suggested the possibility of maintaining a reserve stock of bulbs for each accession that could be planted in the second year.

The European field collection of short-day Allium *species, Israel*

H. Rabinowitch reminded the Group that the collection is maintained in Rehovot at the experimental farm of the Faculty of Agricultural, Food and Environmental Quality Sciences and is supported by the Israeli Gene Bank (IGB).[2] The collection includes garlic, elephant garlic, shallot and *A. tuberosum*. The latter two species are propagated vegetatively to maintain the unique phenotypic quality of each accession. The collection contains a large proportion of the existing germplasm from the short-day zone, including germplasm from Southeast Asia and South America. Collecting missions focusing on the intermediate zone of Central Asia have been ongoing for 5 years, thanks to support from a philanthropic foundation. In particular, 113 accessions collected in 2000 in Central Asia (in collaboration with Rina Kamenetsky and Furkat Hasanov) were all duplicated and sent to Chris Kik in the Netherlands. The collecting mission to Central Asia revealed severe dangers for the local germplasm, since China is flooding the area with cheap garlic, thereby severely threatening business for local garlic producers. Landraces are disappearing at a rapid pace and extensive gathering from the wild for local consumption are not sparing even protected species, such as *A. motor* in Uzbekistan.

H. Rabinowitch mentioned that the cost of maintenance of the collection in Rehovot is very high. The intensive manual work also includes mole control, where the only solution is to bury a rust-protected metal net at a 25-cm depth in order to protect the bulbs from these pests. Moreover, due to continuous build-up of soil-borne pests and diseases, fields have to be relocated. Storage takes up a large amount of space, while foliar and soil-borne diseases require constant control. However, the main problem for the collection remains the financial resources, since the annual support of US$10 000-12 000 from the IGB is insufficient and has to be complemented by research programme funds. Without additional support it will not be possible to increase the collection and its long-term maintenance is also at risk.

Discussion

In reply to questions from the audience, H. Rabinowitch specified the following:

- Considering a cost of US$10 per year per accessions, an external additional support of US$15 000-20 000 per annum would be sufficient to guarantee continued maintenance of the collection, since this amount would be matched with US$10 000-12 000 from Israel.
- The collection is partly evaluated for dry matter content and a few other characters, thanks to special projects; however, in general there is no plan for systematic characterization, due to the limited resources available.

[2] See also: The European field collection of short-day *Allium* species. P. 9 *in* Report of a Working Group on *Allium*, Sixth meeting, op. cit.

- The collection of ornamental *Allium* maintained by Rina Kamenetsky at the Agricultural Research Organization, Volcani Centre, is sustained for a limited period by a charitable fund, not by the Israeli Genebank.

Collection of vegetatively propagated onions in the Nordic Countries

G. Poulsen explained that the Nordic Gene Bank (NGB) only conserves material belonging to the Nordic Countries. The collection includes shallots and potato onions. The responsibility for maintenance lies with the national governments, although so far only Denmark has formally accepted this principle. The material, with a total of 178 accessions mainly consists of potato onions in Finland and Norway, and shallot in Denmark and Sweden. The collections are conserved in clonal archives in their respective countries.

The maintenance of the vegetatively propagated onions is very labour-intensive and the susceptibility to diseases places the collection in constant danger. NGB has therefore established an *in vitro* base collection as a safety-duplication measure, and it is also responsible for the documentation of the collections.

Further collecting of vegetatively propagated onions is recommended in the Nordic Baltic region, as well as rationalization of the collections by identification of duplicates and the elaboration of a characterization system for the potato onion group. Research interests focus on flowering physiology and detection of gene resistance.

Discussion

In the discussion that followed the presentation, G. Poulsen specified that it is the individual countries' responsibility to maintain the clonal archives, as part of the Convention on Biodiversity commitment. NGB's responsibility is to hold information on the material and to secure that all accessions are safety-duplicated. The establishment of the *in vitro* storage facility located at NGB now facilitates this task.

The German vegetatively propagated Allium *collection*

J. Keller reported that 1120 vegetatively propagated *Allium* crop species accessions from all over the world are maintained in the field in the Gatersleben genebank. Garlic, vegetatively propagated *A. ampeloprasum*, chives, bunching onions, *A. tuberosum*, *A. chinense*, some wild species, top onions and other hybrids are replanted every 5 years, while shallots are replanted every year. The field collection also includes 151 safety-duplicates from the Czech Republic (105), Spain (25) and Poland (21), which are permanently cultivated but not available for exchange. A further 1865 accessions of wild species are part of the taxonomy collection (see p. 36), which is now associated with the genebank. Activities for *in vitro* conservation of vegetatively propagated material started in 1990 and cryopreservation research in 1997 (see p. 75). As part of the EU-funded GEN RES project CT95-20 (1996-2000), IPK established a European Core Collection of 25 accessions, embedded in a priority subset of 100 accessions. Furthermore, a virus-free *in vitro* collection consisting of 98 clones has been established.

Vegetatively propagated Allium *genetic resources in Poland*

T. Kotlińska reported that field collections of vegetatively propagated *Allium* germplasm are maintained in two locations by the Research Institute of Vegetable Crops (RIVC). The garlic collection, with 275 accessions, is maintained in Krzczonów. The reduction in the number of accessions was due to losses following unfavourable winters. Passport data are documented for the whole collection and 14 minimum characterization data have been recorded for 259 garlic accessions and sent to the European *Allium* Database (EADB). Additional evaluation data and visual digital documentation are also available. A collection of 139 shallot landraces, mainly from Poland, is conserved in Skierniewice and is fully documented with passport and evaluation data. Thirty-five shallot accessions are conserved

as seed samples. Another fully documented collection of edible wild species (240 accessions from Central Asia, Poland and Siberia) is also maintained at Skierniewice, including species that are strictly or preferentially propagated vegetatively. A small part of the collection is safety-duplicated (70 garlic accessions in Olomouc, Czech Republic; 10 shallot accessions in Wageningen, the Netherlands; and 21 garlic accessions in IPK, Germany).

In connection with the garlic germplasm collection, research on cryopreservation is ongoing, in collaboration with IPK, Germany. Collecting missions in different regions of Poland and neighbouring countries are organized every year.

Plans for the future include increased use of *in vitro* and cryopreservation techniques, extended safety-duplication of samples in different genebanks, increased virus elimination, improved taxonomic identification, continuing collecting missions and improved germplasm characterization, including modern methods.

Discussion

In reply to questions from the audience, T. Kotlińska specified the following:

- Material derived from collecting missions is duplicated in more than one institute; however, this material cannot always be considered formally safety-duplicated, especially if it is not stored within Europe.
- Since 1986, the garlic field collection diminished from 495 to 275 accessions. This was partly the result of the elimination of nearly 100 accessions that were recognized as duplicates. The remaining reduction was due to accessions that were mainly lost during the winters of 1994 and 1996. In normal years, there is no significant loss of accessions in the field.
- No distinction is made in the collection between shallots and potato onions, since no clear differences can be detected.
- All 139 shallot accessions are maintained in field collection every year. Some of these accessions produce large amounts of seed. The seed obtained in isolation is conserved and used for comparison studies and as a safety-duplication measure.
- Regarding the future of the collection, it is positive to consider that it is part of the Polish national programme. However, the yearly budget is uncertain and is subject to frequent cuts. Financial support received in the past from a private breeding company will not be continued, since the company has closed down.

Current status of the Allium *collections in France*

V. Chovelon informed the Group that vegetatively propagated alliums are conserved at two INRA stations in France. INRA-Ploudaniel maintains a field collection of 43 shallot landraces and varieties, mainly from France and the Netherlands, with its own resources. These are mostly virus-infected, but kept free from fungal and bacterial diseases. The accessions are replanted every year. A few more *Allium* species, including tropical shallots, grey shallot and 13 clones of *A. sativum* var. *longicuspis* from Central Asia (Etoh collection) are conserved and used in crossing experiments. The second station, INRA-Monfavet, maintains a garlic collection of 82 accessions (French traditional cultivars, European and tropical garlic accessions, garlic and *A. sativum longicuspis* from the Etoh collection and other *Allium* species). Different activities and research work are carried out on this collection, including maintenance, description, evaluation, virus eradication and selection of interesting accessions.

Portuguese collection

A report on the status of vegetatively propagated *Allium* collections in Portugal was received from R. Farias after the meeting. She reported that cultivated material is maintained in the field in different regions of the country, while wild material is kept at the Banco Português

de Germoplasma Vegetal, Braga, in pots or bunches under a greenhouse. The collection mainly includes *A. sativum* landraces (292 accessions).

United Kingdom collection

D. Astley informed the Group that the only significant collection of vegetatively propagated *Allium* in the United Kingdom is the collection of wild *Allium* species maintained at the Royal Botanic Gardens, Kew (RBGKew). RBGKew holds two collections, one developed by the Jodrell Laboratory (Science Support) mainly for cytotaxonomic research, but also used for anatomical, biochemical and molecular studies. This collection was greatly expanded in the early 1990s for Brian Matthew's study for his "Review of Section *Allium*." The second collection at RBGKew is maintained by the Alpine and Herbaceous Department (under the direction of Tony Hall) and is also available for research purposes. Some of the more decorative species from this collection are utilized in the public alpine display houses. The majority of accessions are maintained in pots on benches or plunged into sand, and both collections are protected from the weather by an open-sided glass roof.

The person formally responsible for *Allium* at RBGKew is Nigel Taylor, curator of the Living Collections Division and Head of the Horticulture and Public Education Department. The two *Allium* collections, which are complementary to the IPK collection, include 761 accessions of mostly wild source living material, generally with good field data. Additionally, well-documented vouchers are also conserved at the Kew herbarium.

Considering that one of the priority areas at RBGKew is monocotyledons, it is unlikely that the *Allium* collection would be under threat in the foreseeable future. D. Astley confirmed that the passport data relating to both collections are being prepared for inclusion in the European *Allium* Database (EADB).[3]

Discussion

D. Astley specified that material would only be available from RBGKew if users complete the RBGKew Material Transfer Agreement. It has been agreed that once the RBGKew data are included in the EADB, a footnote in the "Contributors" file will direct all requests via the HRIGRU (Genetic Resources Unit, Horticulture Research International). However, as very few plants per accession are maintained, requests would only be fulfilled where material surplus to RBGKew's requirements exists. Any bulbs would only be made available at the time of repotting.

The Allium *collections at Plant Research International, The Netherlands*

C. Kik reported that there are two large *Allium* collections at Plant Research International, Wageningen: the freely accessible collection of CGN, and the private collections of the Business Unit (BU) Genetics and Breeding. He went on to describe the BU collections, consisting of onion, leek, garlic and ornamental *Allium* collections. The onion collection, mostly maintained as seeds, consists of onion and its wild relatives, interspecific hybrids, advanced breeding populations and transgenics. The small ornamental *Allium* collection comprises a number of species from the subgenus *Melanocromyum*. The newly formed garlic collection includes about 300 accessions not well characterized yet. The leek collection is subdivided between the so-called von Bothmer collection, which consists of species from the *ampeloprasum* complex, and the leek and wild relative collection. The von Bothmer

[3] Following a visit of D. Astley to Kew Gardens in June 2001, an Excel file was provided for inclusion in the EADB with passport data of the accessions of *Allium* for which the Kew Gardens have living material. The file contains only accession numbers and taxonomic names. Further information will be sent in due course, including country of collection. However, location data will remain protected because of the serious problem of bulb collecting from wild populations for direct marketing or for bulking-up for direct sale. For any *bone fide* interest it will be possible to get information on individual taxa in more detail.

collection, originally collected by R. von Bothmer mainly in the Greek islands, was acquired in 1982. During 19 years of vegetative maintenance, a large number of B-numbers (sites of collection = accession) were lost. The collection was genetically fingerprinted in 1995 with RAPD markers and this permitted a reduction in the number of plants from 2165 to 571, maintaining most of the genetic variation present in the collection. The considerable amount of variation found in the collection offers a high potential value for the introduction in leek of an F1 hybrid breeding system based on cytoplasmic male sterility. The leek and wild relative collection, consisting of 95 accessions, has shown its value for the presence of white tip disease resistance and thrips resistance genes.

Discussion

In reply to questions from the audience, C. Kik specified the following:

- the privatized collections are not publicly accessible; however, they can be used in a joint project;
- the garlic collection received from Israel will be given back after it has been fingerprinted;
- the collections will probably be secure for 5 years, but it is not possible to forecast their long-term destiny;
- regarding the terms of accessibility to the von Bothmer collection, it would be possible to obtain a few accessions, under Material Transfer Agreement, but they would have to be used for scientific and not commercial purposes. For the introduction of accessions into research initiatives, terms and conditions would have to be discussed on a case-by-case basis.

G. Poulsen commented that it would be wise to establish a bilateral agreement with the country of origin before material from the von Bothmer collection is used in any research project for commercial purposes.

Spanish germplasm data

After the meeting, F. Mansilla provided information on the garlic collection conserved in the genebank at CIFA, Córdoba. Thanks to the GEN RES project and currently with support from INIA (Ministry of Agriculture), 45 virus-free clones were produced, a core collection including 25 garlic accessions from the Spanish genebank was developed together with IPK, characterization work was continued, and data were provided to the EADB.

Bulgarian collection

S. Neykov explained that the *Allium* group collection (garlic, leek relatives and wild species) at the Institute of Plant Genetic Resources (IPGR) in Sadovo consists of 151 accessions, mainly received from Germany, United Kingdom and collected in Bulgaria. The Institute of Vegetable Crops “Maritsa” (IVC) in Plovdiv, maintains a field collection of 140 garlic accessions (100 local and 40 breeding material), mostly of subsp. *vulgare*, and for a small part subsp. *sagittatum*, winter and summer forms. The Experimental Station for Vegetable Crops (ESVC) in Gorna Oryahovitsa maintains a field collection of 203 garlic accessions collected in Bulgaria (mostly subsp. *vulgare*). All accessions are documented with passport data and about 80% of the collection in IPGR-Sadovo have been characterized. The garlic collections at IVC-Plovdiv and ESVC-Gorna Oryahovitsa have been evaluated to a limited extent.

Greek collection

S. Samaras reported on the present status of the Greek *Allium* wild taxa collection maintained in the Greek Gene Bank, Thermi-Thessaloniki. In the framework of the EU GEN RES 20 Project, 230 accessions of wild taxa belonging to 38 species have been collected. Seed-producing species are conserved as seed in the Greek Gene Bank. However, due to poor germination and dormancy in most of the wild species, these are being

maintained in pots. It seems that at least for the next 6 years, sufficient resources to ensure the maintenance of these accessions will be available. New collecting expeditions are planned in Greece, aimed at rescuing endangered *Allium* wild relatives.

Germplasm exchange of vegetative alliums—minimum phytosanitary requirements

(Full paper page 66)

A. Senula introduced the issue of the safe movement of vegetatively propagated *Allium*. She gave an account of the most frequently occurring pathogens in vegetative alliums, which may be transmitted with contaminated germplasm (bulbs, cloves and bulbils). The technical guidelines for the safe movement of *Allium* germplasm, published by FAO/IPGRI in 1997,[4] recommend the exchange of *in vitro* material, whenever it is not possible to use seed. *In vitro* plants are free of fungal and visible bacterial infections. Moreover, the establishment of *in vitro* cultures via meristems offers the chance to produce virus-free clones. Research carried out at the IPK genebank showed the possibility of obtaining virus-free *in vitro* plants from virus-infected vegetative plant material. A virus-free *in vitro* collection of garlic at present comprises 98 accessions, free from OYDV, LYSV, GCLV, SLV and allexiviruses. Limiting factors of *in vitro* collections remain the genotype-dependent response to *in vitro* culturing, the lengthy process required to obtain *in vitro* cultures and to transfer back to soil conditions, the labour-intensive technique and the moderately sophisticated equipment required. As a general principle, A. Senula suggested that *in vitro* techniques should be employed to conserve valuable collections, such as core collections, in order to maintain these as high quality virus-free base material. On the other hand, movement of traditional vegetative plant parts, combined with minimum quarantine measures, may still remain an acceptable option in allowing fast exchanges.

Discussion

In the discussion that followed, the possibility to always adopt the optimal practice of transferring only *in vitro* material was questioned, considering that much effort is involved in cleaning the sample.

G. Poulsen and J. Keller stressed the need to maintain healthy material to avoid the risk of spreading infections and losing accessions.

On the subject of virus eradication, J. Keller considered that testing for more than five viruses could be possible, but would not be practical; therefore it could be sensible to eliminate the most dangerous viruses and not deal with latent viruses. However, it was mentioned that the ECP/GR Working Group on Potato also tends to try and eliminate latent viruses.

Regarding the use of PCR techniques versus ELISA test for virus detection, it was considered that PCR would be cheaper; however, ELISA is used at IPK because the methodology is well experimented, while there are no resources available to fine-tune a PCR system.

V. Chovelon clarified that, according to French experience, with ELISA it is possible to check for several viruses. However, it is difficult to identify several strains of one virus and it is only possible to be sure of having eliminated the virus for which the strain is available. In general, the impact of different viruses is difficult to study. For commercial purposes, samples are released free of potyviruses. An attempt to eliminate latent viruses with

[4] Diekmann, M., editor. 1997. *Allium* spp. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. N°18. Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome/Research Institute of Crop Production, Prague-Ruzyne.

combined methods was concluded with 20-25% of virus eradication, a result similar to IPK's achievements. This percentage is considered sufficient to eliminate latent viruses from a number of plants per accession, which are a usable basis to establish virus-free *in vitro* clones. The risk of modification of genetic traits after the meristem regeneration was mentioned as a factor to consider when adopting *in vitro* storage.

V. Chovelon also stressed the need to maintain collections under isolation nets to avoid contamination in the field and the high cost of this practice was highlighted by H. Stavělíková, who mentioned the increasing costs of the nets in the Czech Republic.

H. Rabinowitch, however, claimed that virus-free accessions did not exist in field conditions, as shown by Shiboleth *et al.*[5] He added that complete virus cleaning might even risk making the accessions more susceptible to more virulent strains. He thought that overall the cost of introducing *in vitro* or cryopreservation measures would not be affordable for many genebanks. Also, the genotype dependence of the *in vitro* culture ability must be considered.

It was noted that the cost of permanent maintenance under cryopreservation amounts to only US$1 per accession compared to introduction costs which are higher, and that the task of ensuring cryopreservation of a common collection could be undertaken by a country that could afford the cryo facilities on behalf of all parties concerned. This exercise could start with the garlic core collection, provided that the material was guaranteed to be freely available in the long term and that a reliable and practical method to positively identify genotypic variation so as to avoid duplications exists.

The need to screen the whole European collection in order to identify duplications and reduce the size of the collection was expressed. For this exercise, the results of characterization carried out during the GEN RES project could be used, together with additional morphological screening carried out in two locations. Molecular screening via AFLP was also proposed, considering that the whole European collection could be rapidly screened, following the example of the screening of the IPK garlic collection conducted by Plant Research International (PRI) in the Netherlands. The PRI work confirmed the results of Maass and Klass (IPK)[6], who were able to distinguish nine groups after analysis of the same collection with isozymes and RFLPs. The AFLP screening exercise would also offer an example for other crops to follow.

Recommendations

The Group agreed to fulfil all quarantine regulations for the movement of the Allium *samples. It was considered that special efforts to guarantee the maintenance of virus-free germplasm should be limited to important material such as core collections, as it would be impractical for entire collections due to the high cost of virus cleaning and virus-free maintenance.*[7]

The European *Allium* database

D. Astley presented an outline of the EADB, focusing on the subset of vegetatively propagated alliums. He explained the usefulness of updating the existing data in order to make the information available to potential users and to have a useful baseline for

[5] Shiboleth, Y.M., A. Gal-On, M. Koch, H.D. Rabinowitch and R. Salomon. 2001. Molecular characterisation of onion yellow dwarf virus (OYDV) infecting garlic (*Allium sativum* L.) in Israel: Thermotherapy inhibits virus elimination by meristem tip culture. Ann. Appl. Biol. 138:187-195.

[6] Maass, H.I. and M. Klaas. 1995. Intraspecific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers. Theor. Appl. Genet. 91: 89-97.

[7] It is acknowledged that it is currently not possible to obtain virus-free material for genotypes that are recalcitrant to tissue culture.

rationalization. Appropriate queries to the database would allow identification of the amount and the distribution of duplicated accessions throughout the European collections. It would also serve as the reference catalogue to allow definition of Most Original Samples (MOSs) and attribution of responsibilities for maintenance and for safety-duplication. The history of the development of the EADB is summarized by D. Astley on p. 72-73.

Discussion

C. Kik emphasized that molecular screening of the collection would offer additional and reliable information on the extent of genetic diversity, which would be most practical for the rationalization exercise.

Recommendations

It was agreed that updated data on Allium *collections would be sent* ***by 15 June 2001*** *to the EADB manager in the format agreed at the sixth meeting of the Working Group on* Allium *(*Allium *passport data).*

Considering that the current format is available from the Internet (http://www.hri.ac.uk/site2/research/PGB/ecpgr/ecpgr.htm), it was suggested that curators download the file and use it to replace old data with new data.

It was also agreed that data sent to the EADB should preferably be related to accessions available for distribution.

Cryopreservation

(Full papers in Part II)

In vitro *storage and cryopreservation of* Allium *at IPK*

J. Keller summarized the experience of IPK on *in vitro* culture and cryopreservation of *Allium*. He stressed that the main advantages of *in vitro* storage consist in maintaining pathogen-free material and in reducing space requirements. On the other hand, labour requirements remain rather high. He mentioned that it is not possible in all cases to elaborate specific protocols for each genotype. Therefore, compromise protocols need to be used. A collection of 372 *Allium* accessions including 68 safety-duplicates is currently maintained *in vitro* at IPK. He then explained that storage of meristem explants in liquid nitrogen is still in the developmental phase; however this method is potentially the safest and most cost-effective for long-term conservation. In conclusion, he said that both *in vitro* and cryopreservation can be considered as alternative or complementary methods for germplasm conservation, bearing in mind that the risk of latent infection remains during the *in vitro* phases and therefore appropriate measures for safety-duplication have to be maintained. The lengthy process required to recover entire plants from cryopreserved material is also a limiting factor in the case of active collections.

Discussion

In the following discussion, J. Keller clarified that cryopreservation techniques are already applicable on clove explants, although it is not yet possible to obtain the highest rates of regrowth (on average about 70%) with all the genotypes.

The risk of losing diversity due to genetic drift was said to exist only in the case of non-clonal material.

The best options for long-term conservation were considered to be either cryopreservation or field genebanks with field cages to protect against re-infection. After conclusion of the initial phase of development, cryoconservation was said to be the cheapest option. This was shown by giving the example of potato, where the cost of maintenance is

between 50 cents and 2 US dollars per accession per year. *In vitro* conservation was considered a suitable option for medium-term storage, while latent contamination could become a problem in the long term.

Cryopreservation research at RICP, Czech Republic

J. Zamecnik gave an overview of *in vitro* and cryopreservation research at the Research Institute of Crop Production, Prague. He described the protocols used to induce vitrification of *Allium* shoot tips and reported a regeneration rate as high as 82%.

Discussion

In the following discussion, J. Zamecnik clarified that the material was stored in liquid nitrogen for a period of up to one year and that the Czech genebank intends to use the current testing stage for practical conservation purposes.

Regarding the technique adopted at Fort Collins, USA, where material is stored in vapours at -160ºC, J. Zamecnik affirmed that it is easier to control liquid nitrogen rather than its vapours.

Recalcitrant genotypes were said to remain a limiting factor, while hopes rely on a breakthrough in research on vitrification. The time frame required to achieve an applicable system was said to be possibly 4-5 years, which was the time required for the technique developed on potato to come into practice.

It was reiterated that cryopreservation techniques would offer an important safety backup, but would not replace the role of field genebanks as working collections.

Recommendations

- *The Group believed that* in vitro *conservation remained a useful methodology for backup of the collections in the medium term, although persistent risk of contamination would suggest not solely relying on this form of conservation.*
- *The Group agreed that cryopreservation could be a powerful technique for long-term storage and for safety-duplication of the collections. Research should be continued to make the methodology fully applicable, as in the case of potato.*
- *The Group expressed its wish to monitor the progress made in cryopreservation work over the next 3 years in terms of applicability of the system.*
- *It is recommended that IPGRI provide an analysis of the costs of storage with the different methodologies (field,* in vitro *and cryo).*

Management of the European collections

Introduction

D. Astley informed the Group that the Czech Republic and Israel had formally accepted responsibility for maintenance of the international long-day and short-day *Allium* field collections until the end of Phase VI of ECP/GR (2003). He was pleased to note that most national programmes stated that current maintenance of the *Allium* field material is in fair condition, although potential shortfalls in funds were mentioned in the case of Skierniewice, Poland. He invited the establishment of an early warning system to protect the *Allium* field collections from the risk of loss.

Recommendations

- *The Group agreed on a system of early warning, whereby should curators face a problem at national level threatening the conservation of the* Allium *collections, it is recommended that they alert the Chair or Vice-Chair of the Working Group on* Allium *or the ECP/GR Secretariat.*

- *The Group recommended that, at the onset of the next Phase of ECP/GR, member countries make clear commitments regarding the level of inputs in kind they are ready to ensure at national level.*

Proposal for a conservation network

J. Keller suggested that the creation of a network of specialized centres for the conservation of vegetatively propagated *Allium* could help to share the costs of conservation and improve the security and quality standards of the European collections.

According to this proposal, all the different vegetative *Allium* accessions currently conserved in Europe should be identified in order to become part of a "European collection". The responsibility for maintaining crop subsets of this collection could be accepted by two or more genebanks, designated on the basis of climatic requirements of the different crops and of conservation expertise. Therefore, each accession would be maintained in more than one location as a measure of safety-duplication.

A possible subdivision of the responsibility was suggested as follows:

• long-day garlic	Czech Republic, Germany, Poland, Spain
• short-day garlic	Israel
• shallot	Czech Republic, NGB
• vegetative leek and Mediterranean (oceanic) wild species	Greece, Israel, The Netherlands, Spain
• continental wild species, cold-requiring	Czech Republic, Germany
• chives and Chinese chives	The Netherlands, NGB, UK

Recommendations and workplan

Having considered the examples offered by other Working Groups such as *Beta* and Potato, the following mechanism for the operation of the proposed conservation network was suggested:

1. *Collection holders will make a list of the samples that they would accept responsibility for, based on national priorities and willingness of the national programme to provide inputs in kind, and will send this list to the EADB manager.*
2. *Collection holders will inform the EADB manager about their availability to accept responsibility for additional samples to be conserved as safety-duplicates.*
3. *On the basis of available information, the EADB manager will mark as belonging to the "European collection" all the accessions accepted under specific institutes' responsibility. On the basis of available information, the EADB manager will identify samples for which no one or only one institute offered to accept responsibility for maintenance. The matter will be brought to the attention of the Working Group.*
4. *Accepting responsibility for maintenance of a given sample as part of the "European collection" implies that the institute will maintain it until further notice and will make it available upon request under an agreed MTA.*[8] *Maintenance conditions will be in compliance with the quality standard procedures agreed within the Working Group on* Allium.
5. *If at any point an institute should decide not to continue the maintenance of given samples, it will inform the ECP/GR Working Group sufficiently in advance to identify a replacement institute to take over the responsibility.*

[8] S. Samaras expressed some reservations on the possibility for the Greek Gene Bank to guarantee free exchange of the material.

Safety-duplication strategy for vegetatively propagated Allium

J. Keller proposed a strategy for safety-duplication. Since the maintenance of vegetatively propagated material cannot, for technical reasons, be held under a black-box regime, the safety-duplicate collections should be formalized in an alternative way by individual governments and/or institutions, e.g. each institution should define the accessions for which they accept safety-duplication responsibility and send lists to the EADB. Safety-duplicates should be clearly marked in the documentation system of the host institute. The aim of this strategy would be to ensure that each different sample be maintained at least at two different sites.

The safety-duplicates would not be distributed by the host institutions to any third party, other than with prior agreement from the original holder. In the case of requests sent by third parties to the institution holding the safety-duplicate, this institution would forward the request to the original holder of the accession, at the same time informing the requesting institution of the location from where the sample is available for distribution

Phytosanitary standards

The following two levels of phytosanitary standards were suggested for the material conserved and exchanged as part of the "European collection":

1. General (lower) level

Concerns traditional vegetative material of garlic, shallot, top onions and vegetatively propagated accessions of other* Allium *taxa (bulbs, cloves, bulbils, parts of rhizomes, etc.).

The material exchanged should be free of visible diseases such as fungi, bacteria and arthropods. Prior to dispatch, the material should be disinfected by a fungicide (e.g. by a 0.2% solution of benomyl). For phytosanitary details, see Diekmann (1997).[9]

The recipient of such material would bear the responsibility for the level of quarantine precautions taken when the material is planted, maintained and reproduced.

2. Special (higher) level

Concerns vegetative material as defined in 1 above, belonging to "European core collections" and covers mainly garlic and shallot.

The material should be defined by each institution during the development of the general "European collection" and the selection of their core collections. One of the most important parameters is that the material should be original.

Material of level 2 should be produced mainly for use as a base collection to prevent the total loss of the accession. Under these circumstances it would be possible to treat cryopreserved material similarly to black-box material of seeds.

Sanitary prerequisites are at least the same as for level 1. Some of the institutions that are sufficiently equipped technically should accept a mandate to hold *in vitro* and/or cryopreserved core collections of garlic and shallot (in the extreme case an "*in vitro/* cryo-centre" could be established).

In this *in-vitro*/cryo-centre the material could be stored (preferably by means of cryopreservation) after the establishment of virus-free clones. Two options would be possible:

- **Option A**

  The material goes to the storage centre in virus-free conditions in the form of *in vitro* cultures or clean vegetative organs, together with a certificate of the virus-free state.

- **Option B**

  The material goes to the storage centre as untreated material free of visible diseases and will be freed of viruses at the storage centre.

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[9] (reference: see footnote 4, page 8)

With respect to the viruses in question, two levels of cleanliness could be formulated:

1. (lower level): material free of potyviruses and carlaviruses (allexivirus untested);
2. (higher level): material free of potyviruses, carlaviruses and allexiviruses.

Identification of duplicates

In order to allow the identification of the existing genetic diversity and reduce the occurrence of redundant samples among accessions designated as part of the "European collection", the Group agreed on the following:

1. *Thirteen most important traits will be used by European garlic collection holders to complete the characterization of their own garlic material.*[10] *The data should be sent to the EADB manager after the end of the next growing season. These data will provide the background for comparing newly characterized material with the diversity captured in the garlic core collection built by the EU GEN RES project. The data will also help the identification of duplicate accessions.*
2. *A project on AFLP molecular screening of the whole garlic and shallot collections should be proposed and submitted for funding as an ECP/GR module. D. Astley, C. Kik and J. Keller agreed to prepare a concept note* ***by the end of June 2001*** *and to distribute it to the participants for comments and then to the ECP/GR Secretariat for submission to appropriate agencies.*[11]

[10] The thirteen descriptors are the following:

7.1.5	Foliage attitude
7.1.16.1	Outer skin colour of compound bulb
7.1.16.2	Skin colour of the clove
7.1.19	Number of cloves per compound bulb
7.1.20	Bulb structure type
7.1.21	Shape of the compound bulb in horizontal section
7.1.23	100-bulbil weight
7.1.24	Number of bulbils (topsets)
7.1.22	Weight of cloves
7.2.1	Ability to flower
7.2.2	Ability to produce scape
8.1.7	Daylength requirement (1)
8.2.1.1	Time of flowering

The reference numbers are those used in: IPGRI, ECP/GR, AVRDC. 2001. Descriptors for *Allium* (*Allium* spp.). International Plant Genetic Resources Institute, Rome, Italy/European Cooperative Programme for Crop Genetic Resources Networks/Asian Vegetable Research and Development Center, Taiwan.

[11] According to C. Kik, a preliminary estimate of the costs for a project aiming at fingerprinting the European garlic and shallot collections would be in the range of Euros 237 000.

Closing remarks

The Group recommended that the ECP/GR Secretariat produce a publication to document the outcome of the meeting.

It was recommended that the Group explore all possible means to raise funds for the maintenance and operation of vegetatively propagated *Allium* collections.

A visit to the *Allium* taxonomy collection (see paper p. 36) and to the *Allium* field genebank were kindly organized by the local hosts.

The warm hospitality and the efficient organization offered by IPK personnel, including the pleasant tour of 19 May to the Harz Mountains was gratefully acknowledged.

Part Ⅱ. Presented Papers

International *Allium* Collections

Current status of the international collection of long-day vegetatively propagated Allium *species maintained at the Gene Bank Olomouc, RICP-Prague*

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Introduction

The international collection of vegetatively propagated *Allium* species was established in the former Research Institute of Vegetables Growing and Breeding (RIVGB) in Olomouc in 1986. The establishment of this collection followed the recommendation made at the second meeting of the ECP/GR Working Group on *Allium* (Olomouc, 1986), where former Czechoslovakia, and specifically RIVGB-Olomouc was nominated as the site for the international field genebank for long–day vegetatively propagated *Allium* species.

The Genebank in Olomouc is currently part of the Czech national genebank for agricultural crops. Since 1994, from an organizational point of view, it has been affiliated to the Research Institute of Crop Production (RICP) Prague–Ruzyne. The germplasm collection was started at the RIVGB-Olomouc. This institute, established in 1951, was closed in 1994. In May 2001, the vegetatively propagated *Allium* collection in the RICP, Gene Bank Olomouc includes a collection of garlic (*Allium sativum* L.) and one of shallot (*A. cepa* var. *ascalonicum* Backer).

Garlic collection

The garlic collection consists of 641 accessions. The structure of this collection is shown in Table 1. An important part of the collection is represented by old garlic landraces collected in the Bile Karpaty Mountains and in southern Moravia as well as advanced Czech varieties. Wild species and primitive forms of garlic from Central Asia and Siberia were collected during international collecting missions in Central Asia (1988) and western Siberia (1990). The collection has also increased by 3 landraces from Albania and 9 landraces from Morocco, collected by Paul Havránek during his stay in these countries. Another 11 landraces were collected in the Czech Republic, Poland and Slovenia during international collecting missions between 1999 and 2000. New varieties released in the Czech Republic are regularly included in the collection.

Table 1. Structure of the Olomouc garlic collection according to the origin of accessions

Country of origin	No. of accessions
Former Czechoslovakia	163
Former Soviet Union	146
Bulgaria	69
Austria	45
Poland	42
Spain	27
Romania	24
Hungary	23
Portugal	22
Czech Republic	16
France	14
China	9
Morocco	9
Algeria	4
Japan	4
Other	24
Total	**641**

In recent years 32 accessions were received from Austria, 22 accessions from Portugal (1996), 32 accessions from Poland (1996) and 65 accessions from Bulgaria (1997-1998). Regarding safety-duplication, 63 garlic accessions were sent to IPK (Institute of Genetics and Crop Plant Research), Gatersleben, Germany (1994) and 53 accessions to RICP (Research Institute of Crop Production), Piešťany, Slovakia (1999).

Maintenance of the collection

The collection is maintained in the field in Holice, genebank Olomouc. All accessions are regularly regenerated (replanted) every year. Up to now, only part of the collection, consisting of 25 accessions, has been restored to a virus–free state. These virus-free accessions are grown in isolation cages in field conditions.

Availability of accessions

Ninety percent of the accessions are available for distribution, depending on the health of material, the availability in stock and the need for regeneration during the period between harvesting and planting (July–October).

Evaluation status

The garlic collection is evaluated for 28 characters. The frequency of the various states of these characters is given in Table 2. It is planned to record these characters according to the new edition of the Descriptors for *Allium*.[12]

Documentation of the garlic collection

All passport data have been recorded and computerized. The development of the database of evaluation data is ongoing. Currently the database contains evaluation data for 552 accessions, resulting from 3-year observations.

Table 2. Characterization of the Olomouc garlic collection

Character	Character state	Frequency in the collection (%)
Foliage attitude (in early spring)	prostrate	10.1
	intermediate	14.9
	erect	75
Foliage colour (after winter)	yellowish green	0.7
	greenish yellow	7.1
	light green	7.6
	greenish yellow	84.6
Leaf number (April)	low	5.4
	medium	65.6
	high	29
Plant height (April)	very low	0.2
	low	21.2
	medium	60.5
	high	15
	very high	3.1
Plant vigour	weak	4.5
	intermediate	62
	strong	32.1
	very strong	0.5
Scape	absent	47.8
	present	52.2
Scape height	very low	3.5
	low	65.3
	medium	17.7
	high	12.2
	very high	1.4

[12] IPGRI, ECP/GR, AVRDC. 2001. Descriptors for *Allium* (*Allium* sp.). International Plant Genetic Resources Institute, Rome, Italy/European Cooperative Programme for Crop Genetic Resources Networks/Asian Vegetable Research and Development Center, Taiwan.

Table 2 (cont.). Characterization of the Olomouc garlic collection

Character	Character state	Frequency in the collection (%)
Spathe length	very short	7
	short	28.1
	medium	42
	long	18.4
	very long	4.5
Spathe shape	globe	62.5
	ampule	37.5
Spathe colour	light green	35.8
	green	64.2
Umbel - number of hemispheres	1	85
	2	11.8
	1 - 2	0.4
	2 - 3	1.4
	2 - 4	1
	4	0.4
Number of bulbils (topset)	1 - 15	46.9
	≥16	53.1
Bulbils - anthocyanin	present	100
Foliage attitude (June)	prostrate	0.4
	intermediate	36.8
	erect	62.9
Leaf number (June)	low	2.4
	medium	88.4
	high	9.2
Leaf colour (June)	light green	5.4
	green	83.3
	dark green	11.2
Leaf width (mm)	very narrow	18.7
	narrow	45.3
	medium	29
	broad	6.3
	very broad	0.7
Length of the longest leaf	short	34.1
	medium	42.9
	long	20.8
	very long	2.2
Cross-section of leaf	V-shaped	23.4
	even	76.6
Shaft diameter	narrow	26.5
	medium	45.5
	broad	25.5
	very broad	2.5
Bulb shape	flat globe	24.1
	globe	52.7
	high globe	23.2
Bulb structure type	irregular	61.8
	regular	38.2
Number of cloves per compound bulb	low	12.5
	medium	87.1
	high	0.4
Shape of basal plate	concave	12.3
	even	73.6
	convex	14.1
Bulb skin colour	white	1.5
	cream	94.9
	grey - white	3.6
Bulb - anthocyanin in the skin	absent	37.1
	present	62.9
Bulb - skin thickness	thin	88.2
	thick	11.8
Bulb size	small	22.8
	medium	55.6
	large	21.6

Shallot collection

The shallot collection consists of 119 accessions. Its structure is described in Table 3. The main part of this collection is formed by accessions of Scandinavian origin (Finland and Norway). During the 1997 flood in Olomouc, accessions in the field were damaged and 16 of them were irreversibly lost. Damage of the collections due to unpredictable natural disasters confirms the need to ensure their safety-duplication, as well as the need for new methods of long-term conservation. Recently, the shallot collection increased by 4 landraces, which were obtained from local growers.

Table 3. Structure of the Olomouc shallot collection according to the origin of accessions

Country of origin	No. of accessions
Finland	33
Czech Republic	25
Norway	15
India	11
Former Soviet Union	11
Germany	7
France	4
Austria	4
Other	9
Total	**119**

Safety-duplication

Measures to safety-duplicate the shallot collection have been initiated. Safety-duplicates of 20 accessions were sent to IPK, Gatersleben, Germany, in 1994. Another 17 accessions were sent to the Nordic Gene Bank, Grimstad, Norway, in 1995 and 15 accessions were sent to the Plant Genetic Resources Laboratory, Research Institute of Vegetable Crops (RIVC), Skierniewice, Poland in 1997.

Collection maintenance

The shallot collection is maintained as a field collection in special isolation cages. All accessions are replanted every year. Thirty accessions were cleaned from viruses and they are currently maintained separately.

Availability of accessions

About 90% of the accessions are available for distribution, depending on health conditions and multiplication state, in the period between harvest and planting (July–April).

Evaluation status

The shallot collection is evaluated for 13 characters. The results of 3-year observations are summarized in Table 4.

Documentation of the collection

All passport data have been recorded and computerized; evaluation data are gradually obtained and the database is in progress.

Conclusion

Vegetatively propagated *Allium* species are currently maintained as a field collection. In the near future, it is planned to use new methods of long-term conservation such as cryopreservation. Improvement of the material health, i.e. ensuring that it is virus–free, is in progress. Evaluation will focus on morphological description and qualitative characters (dry matter content of storage organ, flavour strength of storage organ, lachrymatory potency of storage organ).

Table 4. Characterization of the Olomouc shallot collection

Character	Character state	Frequency in the collection (%)
Foliage colour	light green	7
	medium green	75.6
	dark green	17.4
Foliage attitude	prostrate	4
	medium	42
	erect	54
Leaf diameter	narrow	7.8
	intermediate	64.4
	broad	27.8
Degree of leaf waxiness	weak	6.1
	medium	73.9
	strong	20
Plant height	low	18.3
	medium	52.2
	high	25.2
	very high	4.3
Plant vigour	weak	6.1
	medium	63.5
	strong	27.8
	very strong	2.6
Shape of mature dry bulbs	flat	0.8
	flat globe	19.2
	globe	12.2
	high globe	1.7
	spindle	24.3
	cylinder	4.3
	rhombic	36.7
	elliptic	0.8
Population uniformity of bulb shape	uniform	60
	variable	40
Bulb skin colour	white	1.8
	yellow	10.4
	light brown	25
	brown	7.8
	dark brown	0.9
	red	11.4
	yellow to light brown	23.5
	reddish brown	19.2
Average number of bulbs per cluster	very low	6.1
	low	33.9
	medium	46.1
	high	7.8
	very high	6.1
Retaining common tunica scales	absent	73.9
	present	26.1
Ability to produce scape	absent	9.6
	present	90.4
Widened scape	absent	65.3
	present	34.7

The European field collection of short-day Allium *species, Israel*

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The *Allium* genepool is currently under considerable threat due to massive commercial collecting of wild plants (including *A. longicuspis, A. motor, A. odorum* and many others) in Central Asia (the primary centre of diversity). In addition, there is an ever-growing influx of cheap garlic from China, which has put many small farmers out of business. Consequently, a large number of invaluable genotypes have disappeared, never to be regenerated again. Hence the urgency to collect the still available landraces.

Four major vegetatively propagated *Allium* species are preserved in the field genebank of short-day *Allium* species in Israel: (1) garlic (*A. sativum*); (2) great-headed garlic (elephant garlic, *A. ampeloprasum* var. *holmense*); (3) shallot (*A. cepa* Aggregatum Group); and (4) Chinese chives (*A. tuberosum*). Israel and its neighbouring countries have been recognized as the secondary centre of *Allium* spp. evolution. The initial work for complete collection of the local wild *Allium* flora was started with the support of the International Board for Plant Genetic Resources (IBPGR), and the Israeli Gene Bank (IGB) has supported this collection since the early 1990s.

The main sources of material in the collection include samples from various international projects (not directly related to genebank activities), gifts and occasional donations from colleagues and friends, and material collected during missions supported by various funds. In the latter missions, samples of the domesticated species were collected in markets as well as in farmers' fields.

The variation within the garlic collection includes *inter alia* bulb and clove size, number of whorls in the garlic bulb, pigmentation, bolting (ranging from non-bolters via semi-bolters to complete bolters), long-, medium- and short-term keeping.

The shallot accessions present a wide range of variation for several characters, such as bolting, date of maturation, number of bulbs/clusters, leaf size, pigmentation, etc. In many cases, the offspring developed from the few bulbs (garlic and shallot) obtained from a single source show quite a variation with regard to distinct morphological and physiological traits, including bolting vs. non-bolting, skin pigmentation, date of maturation, etc.

Accessions of great-headed garlic show higher uniformity than those of shallot and garlic, but large differences occur between genotypes in many economically important traits. Bolting is common, yet all plants are sterile. Accessions vary in leaf and bulb size, date of maturation, date of flowering, etc.

The collection of Chinese chives is the only one that is treated as a perennial crop. It has not been attacked by any pathogens or pests common in Israel (regardless of their location, in close proximity to many susceptible and infected *Allium* plants), except for a mild infection by thrips. They remain green all year round and produce lavishly in the summer. The accessions differ in leaf size (length, width), tenderness, and date of flowering. Dormancy is common in some accessions, but at least one remains green all year round.

The main difficulties include a continuous battle against soil- and air-borne diseases. As for any given pathogen, some accessions are extremely susceptible and thus serve as a source of infestation. Import and export of diseases pose a severe danger to the receiving end, and therefore quarantine measures have to be taken for every collected entry.

Moles are attracted to alliums. These underground/burrowing mammals consume both roots and bulbs and thus pose a threat to any *Allium* collection. The only viable, though expensive solution is to bury a thick, galvanized metal net (chicken run net) at about 25 cm

below the surface, so as to prevent the moles physically from destroying the roots and the stems.

The vegetatively propagated plants have to be harvested annually, cleaned, stored, sorted, and cloves / bulbs should be separated prior to transplanting. This demands manual labour, which is very costly. Also, since maturation is genotype-specific, watering should be regulated separately for each accession—again a manual task.

Last but not least, public funding does not cover the full cost of the maintenance of the collection. Hence, only essential activities are performed, so as to maintain the collection intact. There is ongoing collaboration with Rina Kamenetsky from the Volcani Centre in Bet Dagan, whose activities focus on ornamental *Allium* species. There is a close collaboration between the two institutes in terms of know-how, in special operations and every possible activity related to both collections.

National Collections

Collecting, evaluation and field maintenance of Allium *species in Bulgaria*

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The germplasm collection of vegetatively propagated Alliaceae in Bulgaria is represented by the genera *Allium* (39 wild and 5 cultivated species), *Nectaroscordum* Lindley (wild species), and *Ipheion* Raf. (Ceschmedziev 1989; Ceschmedziev and Neykov 1999).

The collection

The Bulgarian *Allium* collection includes garlic, leek relatives, wild species and others. The genebank at IPGR-Sadovo, maintains 148 *Allium* species accessions (Table 1). The largest part of the collection was received from Germany and United Kingdom or collected during expeditions in Bulgaria. The Institute of Vegetable Crops (IVC) "Maritsa" in Plovdiv maintains a field collection of 140 garlic accessions (100 local and 40 breeding material), mostly subsp. *vulgare*, and for a small part subsp. *sagittatum*, with winter and summer forms.

Table 1. *Allium* species maintained at IPGR-Sadovo

		Mode of reproduction		
***Allium* species**	**No. of accessions**	**Bulb or clove**	**Additional bulbils**	**Seed**
albidum Fich. ex Bieb.	1	-	-	+
altaicum Pall.	6	-	-	+
ampeloprasum L.	3	+	-	+
angulosum L.	3	-	-	+
atroviolaceum Boiss.	1	+	-	+
caesium Schrenk	1	-	+/-	+/-
cernum Roth	5	-	-	+
dictioprasum C.A.M.	1	+	-	+
farreri	2	+	-	+
fistulosum L.	51	-	-	+
flavum L.	1	-	-	+
ledeborianum Roem	4	-	-	+
lineare L.	2	-	-	+
macrostemon Bunge	1	-	+	+/-
montanum F. W.	1	-	-	+
nutans L.	4	-	-	+
obliquum L.	1	-	-	+
petraeum Kar et Kir	1	-	-	+
ramosum Dan	3	-	-	+
rotundum L.	3	+	-	+
rubens L.	2	+	-	+
sativum L. subsp. *sativum*	9	+	-	-
schoenoprasum L.	16	-	-	+
scorodoprasum L.	3	+	+	+
senescens L.	2	-	-	+
sphaerocephalon L.	2	+	-	+
strictum Serach	2	-	-	+
subhirsutum L.	1	+	-	+
tuberosum	7	-	-	+
victorialis L.	1	-	-	+
vineale L.	1	+	+	+
Allium x *proliferum*	7	-	+	-
Total	**148**			

The Experimental Station for Vegetable Crops (ESVC) in Gorna Oryahovitsa holds a field collection of 203 garlic accessions collected during missions in Bulgaria (mostly subsp. *vulgare*).

Characterization/evaluation

All accessions of the mentioned *Allium* species are documented for passport data, stored in database files in the Bulgarian Genebank in Sadovo and a copy is prepared for integration into the European *Allium* Database. About 80% of the collected material in IPGR-Sadovo has been characterized for 32 characters and evaluated according to the international descriptors (Tronicova *et al.* 1980; Astley *et al*. 1982).

At IVC-Plovdiv and ESVC-Gorna Oryahovitsa garlic collections were evaluated with limited characterization data.

Safety-duplication and maintenance

About 70% of the garlic collection from IVC-Plovdiv are duplicated at the Olomouc Gene Bank in the Czech Republic.

The maintenance methods for the collection in IPGR-Sadovo are given in Table 1 above: bulbs, bulbils and / or seed, according to the mode of reproduction.

Workplan

It is planned to continue the *ex situ* conservation of the vegetatively propagated *Allium* collections, as well as their evaluation and regeneration. Collecting missions and measures for *in situ* conservation are also planned.

Breeding activities will focus on disease resistance, winter and spring / summer forms, winter storage and chemical structure.

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Allium *genetic resources at INRA-Ploudaniel, France*

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INRA-Ploudaniel maintains a vegetatively multiplied field collection of landraces and varieties of shallot (*Allium cepa* Aggregatum Group) (Table 1). The only work carried out on this collection is the observation of a few characters (germination rate, foliage colour, leaf erectness, vigour, shape of the bulbs). Most of these genotypes are virus-infected but they are kept free from fungal and bacterial diseases as much as possible. They are planted and harvested each year. The maintenance of this collection is financed by the institute's own budget.

Table 1. Shallot landraces and varieties in the INRA-Ploudaniel collection

Number	Country of origin	Status of sample*	Variety name	Collecting source**	Description
L1	France	3		2.1	Long bulb
L3	France	3		2.1	Long bulb
L4	France	3		2.1	Long bulb
L6	France	3		2.1	Long bulb
L8	France	5	Jermor		Long bulb
L9	France	3		2.1	Long bulb
L10	France	3		2.1	Long bulb
L11	France	3		2.1	Long bulb
L14	France	3		2.1	Long bulb
L17	France	5	Longor		Long bulb
L18	France	5	Kastell		Long bulb
L19	France	5	Trégor		Long bulb
L20	France	3		2.1	Long bulb
L55	France	5	Brétor		Long bulb
L56	France	5	Ploumor		Long bulb
L57	France	5	Vigarmor		Long bulb
L58	France	5	Gouélor		Long bulb
L59	France	5			Long bulb
DL21	France	3		2.1	Half-long bulb
DL22	France	3		2.1	Half-long bulb
DL23	France	3		2.1	Half-long bulb
DL24	France	3		2.1	Half-long bulb
DL25	France	3		2.1	Half-long bulb
DL26	Unknown	?		?	Half-long bulb
DL28	France	5	Mikor		Half-long bulb
DL29	France	5	Arvro		Half-long bulb
R42	The Netherlands	5	Blonde jaune		Round bulb
R43	France	3		2.3	Round bulb
R44	France	3		2.1	Round bulb
R45	France	3		2.1	Round bulb
R46	The Netherlands	5	Santé		Round bulb
R47	The Netherlands	5	Pikant		Round bulb
R48	USA	5			Round bulb
R49	Germany	3		2.3	Round bulb
R50	France	3		2.3	Round bulb
R51	Russia	3		2.1	Round bulb
R53	France	3		2.1	Round bulb
R54	The Netherlands	5	Golden gourmet		Round bulb
R60	France	5	Lyska		Round bulb
R61	France	5	Primalys		Round bulb
R62	France	5	Polka		Round bulb
R63	The Netherlands	5	Red sun		Round bulb
R64	The Netherlands	5	Springfield		Round bulb

* 3 = traditional cultivar/landrace; 5 = advanced cultivar

** 2.1 = field; 2.3 = garden

We also maintain a few tropical shallots (5 accessions), grey shallot and several *Allium* species (Table 2). The grey shallot, *A. oschaninii* and *A. roylei* are used in our shallot breeding programme. The objectives are to introduce new characters (high dry matter content and resistance to fungal diseases) into the Jersey type shallot. The other accessions are cultivated in pots and they are not characterized.

We also maintain a vegetatively multiplied field collection of 13 clones of *Allium sativum* var. *longicuspis* collected by Takeomi Etoh in Central Asia. Different parameters are evaluated on these clones, including pollen fertility. Experimental crosses are also made. This work is carried out in collaboration with INRA-Montfavet.[13]

Table 2. The INRA-Ploudaniel *Allium* collection

***Allium* species**	**No. of accessions**
A. ampeloprasum	1
A. canadense	1
A. chinense	1
A. fistulosum	1
A. oschaninii	1
A. roylei	1
A. tuberosum	1
A. cepa x *A. fistulosum*	2

[13] See following paper by V. Chovelon.

*The garlic collection (*Allium sativum *L.) at INRA-Montfavet, France*

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The garlic collection, established in 1964 by C.M. Messiaen, is located in southeastern France, at INRA-Avignon, in an old and important area of garlic cultivation. The collection is maintained in the field by vegetative multiplication and bulbs are planted and harvested each year. Most of the genotypes of the collection are virus-infected, but free from fungal diseases and nematodes. The maintenance of this collection is financed by the institute's own budget, in spite of the creation of a national garlic network since 1995.

In 2001, the garlic collection included 82 accessions distributed as follows:

- French garlic clones selected from traditional cultivars, still cultivated or abandoned, maintained by INRA since 1960 (Table 1 - 8 clones), or recently collected in private gardens (since 1991) from old abandoned French traditional cultivars (Table 1 - 13 accessions)
- European garlic accessions (Table 2 - 24 accessions)
- Tropical garlic accessions (Table 3 - 10 accessions)
- Garlic accessions from the Far East (Table 4 - 11 accessions) and *A. sativum longicuspis* from Central Asia (Table 5 - 11 accessions) derived from the Etoh collection
- Other *Allium* (Table 6 - 6 accessions).

Table 1. Garlic clones selected from French traditional cultivars *(in italics: selected clones regenerated by meristem culture to obtain virus-free varieties)*

Varietal group				Collecting		
Messiaen	**Maass**	**Traditional cultivar**	**Selected clone**	**source***	**Ref. n°**	**Year**
1	IIb	*Rose de Lautrec*	*RLBT*	2.1		1960
2	IIb	*Rose du Var*	*BR4*	2.1		1967
		Rose de Corse	*Fructidor*	2.1		1960
		Ail du Nord	AN	2.1		1980
		Rose d'Auvergne	*RA*	2.1		1988
		HNB	Corse	2.3	115	1998
		HNA	Corse	2.3	116	1998
		Farimole	Corse	2.3	117	1998
		San Martino	Corse	2.3	118	1998
3	IIc - IId	Blanc Drôme	BD10 - BD6	2.1		1960
		Violet Cadours	*VC6- VC9*	2.1		1967
		Ail de Vendée	VD 14	2.1		1967
		Bretagne 1	Finistère	2.3	104	1996
		Bretagne 2	Morbihan	2.3	105	1996
		Bretagne 98	Finistère	2.3	114	1998
		Neuvic	Corrèze	2.3	107	1998
		Parthenay	Deux Sèvres	2.3	108	1998
		Schott	Bas Rhin	2.3	110	1998
		Lirac	Gard	2.3	111	1997
		ACSO	Garonne	2.1	112	1997
		Rouge Vendée	Vendée	2.3	113	1997

* 2.1 = field; 2.3 = garden

Table 2. European garlic accessions *(in italics: selected clones regenerated by meristem culture to obtain virus-free varieties)*

Varietal group				Collecting		
Messiaen	**Maass**	**Name**	**Origin**	**source***	**Ref. n°**	**Year**
3	IIc, IId	Blanc Italie	Italy	2.1	106	1997
		Rovigo 24	Italy	2.1	119	1964
		Aguilar de la Frontera	Spain	4	1	1993
		Blanco de Ronda	Spain	4	9	1993
		Puygcerda	Spain	2.1	12	1970
		Pag	Croatia	2.1	13	1970
		Bjelac	Croatia	4	83	1993
		Ruzac	Croatia	4	84	1993
	IIc	Gatersleben 821	Georgia	4	120	1995
	IId	Gatersleben 827	Georgia	4	121	1995
	IIc	Gatersleben 834	Georgia	4	122	1995
	IIc	Gatersleben 848	Georgia	4	123	1995
	IId	Gatersleben 853	Georgia	4	124	1995
2	IIb	Cachi (05312/84)	Hungary	4	100	1996
		Vali (05346/84)	Hungary	4	101	1996
		Cigandi 05544/86	Hungary	4	102	1996
		Kadarkuti 0504195	Hungary	4	103	1996
		Aurgazinskg	Russia	4	125	1991
		A9	Australia	2.1	109	1998
1	IIb	Ajo Muso	Spain	4	50	1993
		Chili	Chile	2.1	10	1996
Central		Tadjikistan	Tadjikistan	3	126	1988
Asia		*Tachkent*	Uzbekistan	3	127	1978
		Liban	*Liban*	3	128	1989

* 2.1 = field; 2.3 = garden; 4 = research institute

Table 3. Tropical garlic accessions *(in italics: selected clones regenerated by meristem culture to obtain virus-free varieties)*

Varietal group				Collecting		
Messiaen	**Maass**	**Name**	**Origin**	**source***	**Ref. n°**	**Year**
5	IIa	*Rouge d'Afrique*	Reunion	4	40	1993
		Gela	Sicily	4	54	1993
6	Vb	*Egypte 2*	Egypt	4	42	1993
		Sancti Spiritus	Cuba	4	46	1993
		Ti Vacoa	Reunion	2.1	47	1993
		Rouge Reunion 67	Reunion	2.1	51	1993
		Mexique	Mexico	4	52	1993
		Grandiolais	West Africa	4	30	1993
1 (?)		Niger	Niger	4	31	1993
		Senegal	Senegal	4	32	1993

* 2.1= field; 4 = research institute (from Messiaen collection)

Table 4. Garlic accessions from the Far East

Varietal group			Collecting		
Messiaen	**Maass**	**Name**	**source***	**Ref. n°**	**Year**
3	IIc, IId	Formose	4	59	1993
		Howaïto Nagana (116)	4	62	1993
		Howaïto Iwate (111)	4	72	1993
		Fukushi Howaito (118)	4	73	1993
		Kanchi Howaïto (124)	4	75	1993
		Iwaki shi (120)	4	74	1993
1	IIb	Kagoshima (27)	4	60	1993
		Ambon (74)	4	61	1993
		Shishigahama Zairai (119)	4	63	1993
6	Vb	Thaï 91	4	65	1993
		Fukushi (54)	4	69	1993

* 4 = research institute (from Etoh collection)

Table 5. *Allium sativum longicuspis* collection

Name	Origin	Collecting source*	Year
J130	Moscow	4	1993
J184	Tachkent	4	1993
J190	Samarkand	4	1993
J191	Samarkand	4	1993
J197	Dushambe	4	1993
J200	Frunze	4	1993
J203	Alma Ata	4	1993
J204	Alma Ata	4	1993
J205	Alma Ata	4	1993
J209	Moscow	4	1993
J211	Moscow	4	1993

* 4 = research institute (from Etoh collection)

Table 6. Other *Allium*

Name	Origin	Year
Hexaploïd (*A. polyanthum* x *A. sativum*?)	Unknown	1970
A. ophioscorodon	France (Strasbourg)	1970
A. cepa var. *aggregatum* Griselle	France (Drôme)	1960
A. cepa var. *aggregatum* Griselle "Scalogno di Romagna"	Italy (Univ. Bologna)	1998
A. cepa var. *aggregatum:* DLGK3	France (Brittany)	1980
A. cepa var. *aggregatum*: DLL2	France (Brittany)	1980

Collecting source: 4 = research institute (from Messiaen collection)

Various activities and research works have been conducted on the garlic collection:

- Maintenance, description, classification and partial evaluation of the different accessions following the UPOV descriptors
- Delimitation of varietal groups by morphological and physiological characters checked by isozyme markers (Lallemand et al. 1994)
- Partial evaluation of garlic ecotypes for sulphur compounds
- Selection of interesting accessions, regeneration by meristem culture and clonal selection, obtention of virus-free varieties
- Study of sexual multiplication: over the past 4 years important work has been carried out on the Allium sativum longicuspis collection (Table 5). Different parameters (climate, pollen fertility, pollination methods, seed maturity and germination rate) are evaluated for seed production and experimental crosses are carried out.

The list of varieties listed in the French Garlic catalogue is given in Table 7.

Table 7. Varieties listed in the French Garlic catalogue

Varietal group (Messiaen)	Origin	Traditional cultivar	Selection*	Variety name	Obtained by	Date
3	France	Blanc Drôme	BD 10 (CS)	Messidrôme	INRA	1973
			BD 6 (CS)	Thermidrôme	INRA	1973
	France	Violet Cadours	VC6 (CS + MC)	Germidour	INRA	1979
	France	Blanc Lomagne	(CS + MC)	Corail	CEFEL	1992
			(CS + MC)	Jolimont		1992
	France	Blanc Issoudun	IS (CS + MC) Is (11-12-15)	Mondor	INRA	1998
	Spain	Blanco Ronda	BR (CS + MC) Br (14-19)	Novatop	INRA/TopSemence	1996
	USA	unknown	unknown	VigoSuprem		1995
				Vigor Max		1997
2	France	Moulinen	(CS + MC)	Moulinor	INRA	1992
	France	Rose Corse	Fructidor (CS + MC)	Printanor	INRA	1983
	France	Rose Auvergne	RA (CS + MC) Ra (1-3-4)	Clédor	INRA	1997
	France	Ail du Nord	unknown	Artop	TopSemence	1992
	France	unknown	CS + MC	Cristo	CTIFL	1992
	France	Ail du Nord	CS	Gayant	Artois bulb	1992
	Italy	Rose Rovigo	Ro24 (CS + MC) Ro (2-6-8)	Flavor	INRA	1998
1	France	Rose Lautrec	RLBT (CS + MC) IB (7-16)	Ibérose	INRA	1994
			GL (CS + MC) Gl (4-10-17)	Goulurose	INRA	1994
	Spain	Morado	CB (MC) Cb 23	Morasol	INRA/TopSemence	1994
			Ag (MC) Ag 3	Moraluz	INRA/TopSemence	1996
			C (MC) C 12	Moratop	INRA/TopSemence	1996
	Italy	Rouge sulmona	S (MC) S(1)	Sultop	INRA/TopSemence	1994
	Algeria	Violet Kabylie	Kb (CS + MC) Kb(1-5)	Morasur	INRA/TopSemence	1994
	Argentina		(MC) Arg 20	Edenrose	INRA	1998
			(MC) Arg 17	Jardirose	INRA	2001
	Uzbekistan	Tachkent	(MC) F(2-10-12)	Sprint	INRA	1991
	Kazakstan	Alma Ata	(MC) 204-15	Maxitop	INRA/TopSemence	1999
	Liban	Liban 95	(MC) Lb(1-6-8)	Primor	INRA	1997
5	Egypt	Blanc d'Egypte	Ineg 5 (CS + MC) Eg(2-3)	Ramsès	INRA	1994

***** CS = clonal selection; MC = meristem culture

Reference

Lallemand, J., C.M. Messiaen, F. Briand and T. Etoh. 1997. Delimitation of varietal groups in garlic (*Allium sativum* L.) by morphological, physiological and biochemical characters. First Symposium on Edible Alliaceae, March 1994, Mendoza, Argentina (J.L. Burba and C.R. Galmarini, eds). Acta Hort. 433:123-132.

German collections of vegetatively propagated Allium

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There are two large genebanks in Germany. The Braunschweig genebank presently maintains 79 accessions of *Allium*, whereas the Gatersleben collection is much larger, amounting to 1571 accessions. No vegetative material is maintained in Braunschweig, whereas a considerable part of the accessions, namely 922 (i.e. 59%) has to be vegetatively maintained at Gatersleben. A further 1865 accessions constitute the wild species collection. The main steps of acquisition of the collected material and the structure of the Gatersleben collection is determined by the following factors:

- The collection has been permanently sampled since 1950 (first shallot, first top onion) and 1953 (first garlic) respectively, together with a very broad spectrum of other species. Thus, the alliums have been embedded in the formerly so-called "Kulturpflanzen-Weltsortiment" (= crop plant world collection) as part of a multicrop collection dedicated to crop plant research and as a holding for use by researchers and breeders.
- During the existence of the German Democratic Republic (GDR), several calls for accessions were made around 1975 to collect the material of home gardens. As a result, a considerable number of garlic, shallots, top onions and pearl onions were collected from the East German territory.
- Especially well-developed connections were established with the former Soviet Union, with the most interesting locations in Central Asia and the Caucasian region, as well as with China, Cuba, Italy, and North Korea. Therefore, the genebank contains a high percentage of material originally sampled in these regions.
- Mainly between 1985 and 1995, a research project was active in the Taxonomy Department at IPK-Gatersleben where a special research collection of very high value, owing to its completeness with respect to material and information, was established. Most of these accessions have to be treated as perennials, hence as vegetatively propagated material. This collection is now associated to the genebank.[14]
- Taking into account that the vegetatively propagated alliums are more endangered by diseases than are the seed-forming materials, *in vitro* culture activities were started in 1990 and research on cryopreservation was initiated in 1997 with the support of an IPGRI Special Project.[15]
- Vegetatively propagated alliums were a substantial target of the EU-funded GEN RES Project CT95-20, running from 1996 to 2000, during which IPK concentrated on garlic, resulting in the establishment of a European Core Collection of 25 accessions embedded in a priority subset of 100 accessions. Furthermore, a virus-free *in vitro* collection was established.[16]

At present, the following numbers of accessions are permanently cultivated in the genebank field: garlic (486); vegetatively propagated forms of *A. ampeloprasum* s.l. (49); chives (17); bunching onion (79); *A. tuberosum* (23); *A. chinense* (2); wild species (73); top onions (151); and other hybrids (128). These groups are replanted every five years. The 112 shallots are replanted every year. This gives a total of 1120 accessions. As a consequence of the GEN RES Project, a special cultivation plot consisting of 73 accessions, including the northern part of the European Core Collection, is under the supervision of the

[14] See following paper by R. Fritsch, p. 36.
[15] See paper by J. Keller and A. Senula, p. 75.
[16] See paper by A. Senula and J. Keller, p. 66.

"*In vitro* Storage Group". IPK's vegetative *Allium* collection comprises 141 safety-duplicates from the Czech Republic (105), Spain (25), and Poland (21), which are permanently cultivated but not available for exchange. A further 21 accessions from Spain are still under preparation by the "*In vitro* Storage Group" for inclusion in the genebank safety-duplicates collection.

A short history of the taxonomic collection of genus Allium *housed at the Institute of Plant Genetics and Crop Plant Research (IPK)*

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More than two decades ago, the serious need for detailed investigation of the taxonomic identity and systematic placement of cultivated, semi-cultivated, as well as exploited wild *Allium* species initiated the start of a special research programme at the then Zentralinstitut für Genetik and Kulturpflanzenforschung at Gatersleben. According to the traditions of Gatersleben taxonomic research, in 1980 the creation of a comprehensive living collection was initiated (Figs. 1 and 2), to act as a basis for the research activities. From its very beginning, this special collection was intended to complement and not to compete with the already existing rich genebank collection of cultivated *Allium* accessions. Therefore the taxonomic collection should present a wide array of infraspecific as well as infrageneric diversity of wild species to be the main target of research, to which cultivated strains could be added according to necessity.

Sources of the taxonomic collection

The construction of the taxonomic collection exploited four main sources:

1. *Allium* seed offered for exchange by Botanical Gardens. It soon became evident that not more than about 80 taxa could be received from this source, although germination ability and accuracy of taxonomic determination were below expectations. Some species like *A. turkestanicum* and *A. libani* were never correctly named if obtained from botanical gardens. Plants were often discarded since they did not correspond to the taxa attributed by the donors. Initially much exploited, this source of material was later only used if no other possibility was available. Our data show that about 80% of the *Allium* species offered by certain European botanical gardens were mis-determined (but some gardens on the other hand regularly offered well determined material).
2. Living plants or seeds from other scientific collections, either to serve as well-characterized references ("standards") for taxa of different levels, or to be the object of further research. For example, we received material from the Gothenburg Botanical Garden from the southwestern Asian collections left by Per Wendelbo; several accessions from the Kew Gardens special *Allium* collection; and gifts from very many institutional as well as private breeding stations we cannot list here individually.
3. Living plants or seeds collected by co-workers and friends during excursions, holidays, scientific conferences, etc.
4. Living plants or seeds collected in the wild during special research missions in different countries. In this way a sub-collection of "taxa from their type locations" was established, which is indispensable for qualified and careful taxonomic decisions. These missions were jointly undertaken with botanical research institutions in different countries.

A review of these activities is presented in Table 1 below.

Fig. 1. The field area in 1996.

Fig. 2. Detail of the field area.

Table 1. Collecting missions which contributed materials to the collection

Year	Destination	Participants from IPK	Cooperating institutions
1980	Italy	P. Hanelt, K. Hammer	Istituto del Germoplasma, Bari
1981	Bulgaria	P. Hanelt	Bot. Institute, Bulgarian Acad. Sc., Plovdiv
1981	Slovakia	K. Pistrick, H. Ohle	Agricultural Institute, University of Brno
1981	Georgia	P. Hanelt, J. Kruse	Bot. Institute, Georgian Acad. Sc., Tbilisi
1981	Italy	K. Hammer, Chr. O. Lehmann	Istituto del Germoplasma, Bari
1981	South Italy	K. Hammer	Istituto del Germoplasma, Bari
1982	Georgia	P. Hanelt, R.M. Fritsch	Bot. Institute, Georgian Acad. Sc., Tbilisi
1983	Tajikistan	P. Hanelt	Bot. Institute, Tajik Acad. Sc., Dushanbe
1983	Italy	K. Hammer	Istituto del Germoplasma, Bari
1983	Georgia	R.M. Fritsch, K. Pistrick	Bot. Institute, Georgian Acad. Sc., Tbilisi
1983	Italy	K. Hammer	Istituto del Germoplasma, Bari
1984	Tajikistan	R.M. Fritsch	Bot. Institute, Tajik Acad. Sc., Dushanbe
1984	Georgia	P. Hanelt, J. Schultze-Motel	Bot. Institute, Georgian Acad. Sc., Tbilisi
1984	Italy	K. Hammer	Istituto del Germoplasma, Bari
1985	Mongolia	P. Hanelt, J. Kruse	Bot. Institute, Mongolian Acad. Sc., Ulan-Baatar
1985	Georgia	R.M. Fritsch, K. Pistrick	Bot. Institute, Georgian Acad. Sc., Tbilisi
1985	South Italy	K. Hammer	Istituto del Germoplasma, Bari
1986	Iraq	K. Hammer	Agriculture & Water Research Centre, Baghdad
1986	Tajikistan	R.M. Fritsch	Bot. Institute, Tajik Acad. Sc., Dushanbe
1986	North Korea	P. Hanelt	Bot. Institute, Korean Acad. Sc., Pyongyang
1986	Georgia	P. Hanelt, K. Pistrick.	Bot. Institute, Georgian Acad. Sc., Tbilisi
1987	Tajikistan	R.M. Fritsch	Bot. Institute, Tajik Acad. Sc., Dushanbe
1987	Bulgaria	J. Kruse, J. Schultze-Motel	Bot. Institute, Bulgarian Acad. Sc., Plovdiv
1987	Mongolia	K. Pistrick	Bot. Institute, Mongolian Acad. Sc., Ulan-Baatar
1988	China	P. Hanelt	Botanical Institute, Kunming
1988	Central Asia	R.M. Fritsch	Bot. Institutes Dushanbe & Tashkent
1988	Georgia	K. Pistrick	Bot. Institute, Georgian Acad. Sc., Tbilisi
1989	Georgia	P. Hanelt	Bot. Institute, Georgian Acad. Sc., Tbilisi
1990	Central Asia	R.M. Fritsch, K. Pistrick	Bot. Institutes Dushanbe Tashkent Alma-Ata
1991	Tajikistan	R.M. Fritsch	Bot. Institute, Tajik Acad. Sc., Dushanbe
1991	Altai	K. Pistrick, N. Friesen	Siber. Bot. Garden Russ. Acad. Sci., Novosibirsk
1992	Tunisia	K. Pistrick	Institut des Régions Arides, Médenine
1992	Central Asia	R.M. Fritsch	Bot. Institute, Uzbek Acad. Sc., Tashkent
1993	Italy	K. Hammer	Istituto del Germoplasma, Bari
1993	Tunisia	K. Pistrick	Institut des Régions Arides, Médenine
1993	Central Asia	R.M. Fritsch	Bot. Institute, Uzbek Acad. Sc., Tashkent
1993	Albania	K. Pistrick	FAO
1993	Italy	K. Hammer, Th. Gladis	FAO / Istituto del Germoplasma, Bari
1994	Iran	R.M. Fritsch	Plant Pests & Diseases Res. Institute, Tehran
1994	Central Asia	R.M. Fritsch, K. Pistrick	Bot. Institute, Uzbek Acad. Sc., Tashkent
1995	Central Asia	R.M. Fritsch	Bot. Institute, Uzbek Acad. Sc., Tashkent
1995	Turkey	N. Friesen, R.M. Fritsch	Dept. Pharm. Botany, Istanbul Univ., Istanbul
1995	Central Asia	K. Pistrick	Bot. Institute, Uzbek Acad. Sc., Tashkent
1997	Central Asia	R.M. Fritsch	Bot. Institute, Uzbek Acad. Sc., Tashkent
1998	Central Asia	N. Friesen, R.M. Fritsch	Bot. Institute, Uzbek Acad. Sc., Tashkent

About 1300 accessions were contributed from these missions, but about 230 accessions died prior to determination. Approximately 500 accessions were added from the collecting activities mentioned above (points 2 and 3). Although about 55% of all accessions ever included into the documentation came from botanical gardens, less than 20% of the definitely determined species and subspecies which became part of the collection stem from this source.

Maintenance and development of the collection

The plants were cultivated at three different cultivation areas: pots placed in a frame were used to grow plants from seed till flowering. Frost-susceptible species were permanently grown in pots and were put in a cool glasshouse during winter. Pots were also used for taxa

not thriving well under field conditions, or for those demanding special treatment. Raised beds were later contructed to cultivate taxa from arid countries protected from the rain, but otherwise plants were subjected to local weather conditions.

Thus the collection developed steadily. In 1985, the "magic" number of 80 definitively determined species was reached. Since 1987, when the documentation changed to electronic media, the development can be demonstrated as follows (Table 2).

Table 2. Collection dynamics since 1987 (numbers of definitively determined accessions, species and subspecies were counted). Till the mid-1990s subgenus *Bromatorrhiza* was recognized but is not represented in this table

Year	No. of taxa	No. of access.	Subgenus *Allium*		Subgenus *Amerallium*		Subgenus *Calo-scordum*		Subgenus *Melano-crommyum*		Subgenus *Rhizirideum*		Other genera	
			Taxa	Acc	Taxa	Acc	Taxa	Acc	Taxa	Acc	Taxa	Acc	Taxa	Acc
1987	175	760	59	231	26	83	1	2	17	45	70	371	2	11
1988	191	936	63	305	27	90	1	3	24	69	73	437	3	14
1989	205	1096	65	342	28	97	1	3	27	131	80	486	4	16
1990	220	1173	67	358	32	110	1	3	31	149	83	505	6	18
1991	247	1332	72	395	35	124	1	1	40	200	91	556	8	22
1992	273	1444	70	405	41	132	1	2	46	236	103	599	12	27
1993	297	1588	80	447	46	148	1	2	52	266	105	667	13	27
1994	327	1758	90	503	57	175	1	3	56	316	108	698	15	30
1995	365	1977	98	578	64	196	1	6	66	358	116	770	19	34
1996	384	1771	107	557	67	186	1	6	70	321	117	624	21	37
1997	390	1842	111	592	69	206	1	5	70	324	117	631	21	41
1998	396	1860	112	586	68	210	1	4	74	345	118	630	22	42
1999	373	1688	102	517	64	200	1	4	74	318	109	591	22	42
2000	384	1865	106	540	64	202	1	5	82	330	107	621	23	45
1987-2000	**449**	**3157**	**132**	**929**	**74**	**278**	**1**	**9**	**88**	**522**	**130**	**1333**	**23**	**60**

The most active *Allium* research phase was roughly between 1985 and 1995. In the mid-1990s the field part of the collection occupied so much space that adequate handling became difficult and some reduction became necessary. Therefore the number of accessions was reduced in the most accession-rich species. According to our data, about 350 *Allium* species and not much more than 30 species of closely related genera can be cultivated at Gatersleben. This stage was reached about 5 years ago. During the last 5 years about the same number of species as was added has been lost.

The above-described cultivation areas are still in use today, although the field area in particular has shifted to some extent. Most species of subgenus *Rhizirideum* set seed every year, but especially the taxa from arid areas set seed only in exceptionally dry and hot summers. There is strong evidence that many species freely intercross with any other species present nearby and set seed, but the germination of this seed is low and most seedlings do not survive. Therefore, at present most species are vegetatively preserved and careful selection is made if accessions are re-established from seed. The production of true-breeding seed is rather expensive because complete isolation from other flowering accessions and adequate presence of pollinators must be warranted. Some trials using isolation cabins resulted in low seed yield.

Most recently, the main research activities of the IPK Taxonomy Department have shifted away from *Allium*. Thus the collection cannot be held and managed any longer as a research collection, although it remains the largest living taxonomic *Allium* collection worldwide with eminent importance. Now the *Allium* collection has become part of the special collections held by IPK under the supervision of the Genebank Department. The collection is named "Taxonomic *Allium* Reference Collection" and is horticulturally managed by a garden service under taxonomic supervision by the Taxonomy Department.

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Report on the current status of the Greek Allium *wild taxa collection maintained at the Greek Gene Bank*

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In the framework of the EU GEN RES 20 Project, over 200 accessions of wild taxa belonging to 35 species were collected (Table 1). Many of these were planted in pots to be characterized and maintained as living material as well as for further research (Table 2). Some species of the material collected showed a great diversity since they were found in habitats ranging from sea level up to 1000 m. Some were endemic and found only in a distinct habitat. Accessions producing seed have been conserved in the Greek Gene Bank, but they were found to be difficult to reproduce, due to poor germination and dormancy. It was therefore decided that most of the wild species would be maintained in pots as long as there would be adequate financial support. For the time being, it seems that at least for 6 years there will be no problem in maintaining the accessions in pots, since the Greek Gene Bank has received funds for a large project that will ensure the maintenance of this material. This project also foresees the construction of new premises for the Greek Gene Bank with fully equipped laboratories and new cool and cold rooms for conservation. In addition, collecting expeditions will take place in Greece to rescue as many species as can be found among the wild relatives of the cultivated crops, including *Allium*.

Table 1. Wild *Allium* species collected by the Greek Gene Bank (GRGGB) in the framework of the EU GEN RES 20 Project

Acc. n°	Species	Subspecies	Section
10042	*amethystinum*		Allium
10052	*amethystinum*		Allium
10002	*ampeloprasum*		Allium
10008	*ampeloprasum*		Allium
10017	*ampeloprasum*		Allium
10021	*ampeloprasum*		Allium
10033	*ampeloprasum*		Allium
10051	*ampeloprasum*		Allium
10065	*ampeloprasum*		Allium
10070	*ampeloprasum*		Allium
10072	*ampeloprasum*		Allium
10079	*ampeloprasum*		Allium
10089	*ampeloprasum*		Allium
10092	*ampeloprasum*		Allium
10095	*ampeloprasum*		Allium
10096	*ampeloprasum*		Allium
10098	*ampeloprasum*		Allium
10100	*ampeloprasum*		Allium
10109	*ampeloprasum*		Allium
10126	*ampeloprasum*		Allium
10128	*ampeloprasum*		Allium
10129	*ampeloprasum*		Allium
10130	*ampeloprasum*		Allium
10138	*ampeloprasum*		Allium
10140	*ampeloprasum*		Allium
10142	*ampeloprasum*		Allium
10143	*ampeloprasum*		Allium
10145	*ampeloprasum*		Allium
10146	*ampeloprasum*		Allium
10148	*ampeloprasum*		Allium
10149	*ampeloprasum*		Allium
10150	*ampeloprasum*		Allium
10154	*ampeloprasum*		Allium
10156	*ampeloprasum*		Allium
10158	*ampeloprasum*		Allium
10161	*ampeloprasum*		Allium
10162	*ampeloprasum*		Allium
10166	*ampeloprasum*		Allium
10167	*ampeloprasum*		Allium
10169	*ampeloprasum*		Allium
10171	*ampeloprasum*		Allium
10172	*ampeloprasum*		Allium
10176	*ampeloprasum*		Allium
10178	*ampeloprasum*		Allium
10183	*ampeloprasum*		Allium
10185	*ampeloprasum*		Allium
10186	*ampeloprasum*		Allium
10189	*ampeloprasum*		Allium
10192	*ampeloprasum*		Allium
10193	*ampeloprasum*		Allium
10198	*ampeloprasum*		Allium
10204	*ampeloprasum*		Allium
10205	*ampeloprasum*		Allium
10207	*ampeloprasum*		Allium
10213	*ampeloprasum*		Allium
10221	*ampeloprasum*		Allium
10222	*ampeloprasum*		Allium
10099	*bourgeaui*	*cretica*	Allium
10105	*callimischon*	*aemostictum*	Brevispatha
10004	*caristanum*		
10114	*cepa*		
10173	*cepa*		
10107	*chamaespathum*		Allium
10077	*commutatum*		Allium
10106	*commutatum*		Allium
10101	*cyrili*		Melanocrommyum

Acc. n°	Species	Subspecies	Section
10080	*dentiferum*		Codonoprasum
10084	*dentiferum*		Codonoprasum
10097	*dentiferum*		Codonoprasum
10102	*dentiferum*		Codonoprasum
10180	*dentiferum*		Codonoprasum
10206	*dentiferum*		Codonoprasum
10108	*dilatatum*		Allium
10093	*dodecanesi*		Codonoprasum
10018	*euboides*		
10024	*flavum*	*tauricum*	
10039	*flavum*		Codonoprasum
10043	*flavum*	*flavum*	
10047	*flavum*		Codonoprasum
10054	*flavum*		Codonoprasum
10059	*flavum*		Codonoprasum
10086	*flavum*	*tauricum*	
10087	*flavum*	*tauricum*	
10116	*flavum*		Codonoprasum
10122	*flavum*		Codonoprasum
10123	*flavum*		Codonoprasum
10212	*flavum*		Codonoprasum
10218	*flavum*		Codonoprasum
10090	*goullimyi*		Scorodon
10001	*guttatum*		Allium
10007	*guttatum*		Allium
10012	*guttatum*		Allium
10013	*guttatum*		Allium
10019	*guttatum*		Allium
10023	*guttatum*	*sardoum*	
10025	*guttatum*	*sardoum*	
10029	*guttatum*		Allium
10046	*guttatum*		Allium
10049	*guttatum*		Allium
10053	*guttatum*		Allium
10055	*guttatum*		Allium
10062	*guttatum*		Allium
10066	*guttatum*		Allium
10071	*guttatum*	*sardoum*	
10074	*guttatum*	*sardoum*	
10082	*guttatum*	*sardoum*	
10091	*guttatum*		Allium
10094	*guttatum*	*sardoum*	
10139	*guttatum*		Allium
10141	*guttatum*		Allium
10144	*guttatum*		Allium
10147	*guttatum*		Allium
10151	*guttatum*		Allium
10152	*guttatum*		Allium
10153	*guttatum*		Allium
10155	*guttatum*		Allium
10160	*guttatum*		Allium
10168	*guttatum*		Allium
10175	*guttatum*		Allium
10181	*guttatum*		Allium
10188	*guttatum*		Allium
10191	*guttatum*		Allium
10195	*guttatum*		Allium
10199	*guttatum*		Allium
10200	*guttatum*		Allium
10201	*guttatum*		Allium
10203	*guttatum*		Allium
10209	*guttatum*		Allium
10210	*guttatum*		Allium
10211	*guttatum*		Allium
10217	*guttatum*		Allium
10220	*guttatum*		Allium
10038	*guttatum/vineale*		Allium
10040	*guttatum/vineale*		Allium
10057	*guttatum/vineale*		Allium

Acc. n°	Species	Subspecies	Section
10215	*integerrinum*		Allium
10032	*integgerinum*		Allium
10022	*lagarophylum*		Scorodon
10076	*neapolitanum*		Molium
10083	*neapolitanum*		Mollium
10078	*nigrum*		Melanocrommyum
10081	*nigrum*		Melanocrommyum
10124	*nigrum*		Melanocrommyum
10016	*pallens*		Codonoprasum
10068	*pallens*		Codonoprasum
10088	*pallens*		Codonoprasum
10179	*pallens*		Codonoprasum
10194	*pallens*		Codonoprasum
10011	*paniculatum*		Codonoprasum
10015	*paniculatum*	*vilosullum*	
10067	*paniculatum*		Codonoprasum
10184	*paniculatum*		Codonoprasum
10190	*paniculatum*		Codonoprasum
10197	*paniculatum*		Codonoprasum
10026	*phthioticum*		Mollium
10085	*proponticum*		Allium
10131	*proponticum*		Allium
10132	*proponticum*		Allium
10136	*proponticum*		
10073	*roseum*		Molium
10104	*rubrovittatum*		Allium
10174	*sativum*		
10117	*scorodoprasum*		
10119	*scorodoprasum*		
10120	*scorodoprasum*		
10125	*scorodoprasum*		
10134	*scorodoprasum*		
10003	*sphaerocephalon*		Allium
10005	*sphaerocephalon*		Allium
10010	*sphaerocephalon*		Allium
10030	*sphaerocephalon*	*trachypus*	
10031	*sphaerocephalon*	*trachypus*	
10034	*sphaerocephalon*		Allium
10036	*sphaerocephalon*		Allium
10044	*sphaerocephalon*		Allium
10061	*sphaerocephalon*		Allium
10133	*sphaerocephalon*	*aegeum*	Allium
10163	*sphaerocephalon*		Allium
10164	*sphaerocephalon*		Allium
10177	*sphaerocephalon*		Allium
10208	*sphaerocephalon*		Allium
10216	*sphaerocephalon*	*trachypus*	Allium
10219	*sphaerocephalon*		Allium
10009	*staticiforme*		Codonoprasum
10020	*staticiforme*		Codonoprasum
10118	*staticiforme*		Codonoprasum
10157	*staticiforme*		Codonoprasum
10159	*staticiforme*		Codonoprasum
10014	*subhirsutum*		Mollium
10075	*subhirsutum*		Molium
10103	*tardans*		Codonoprasum
10115	*trifoliatum/ subhirsutum*		Mollium
10027	*vineale*		Allium
10041	*vineale*		Allium
10048	*vineale*		Allium
10050	*vineale*		Allium
10056	*vineale*		Allium
10058	*vineale*		Allium
10063	*vineale*		Allium
10064	*vineale*		Allium

Table 2. Species maintained in pots - Greek Gene Bank, 2001

N°	Section	Genus	Species	Subspecies	No. of accessions	Distribution*
1	Allium	*Allium*	*guttatum*		41	W
2	Allium	*Allium*	*vineale*		17	W
3	Allium	*Allium*	*ampeloprasum*		28	W
4	Allium	*Allium*	*sphaerocephalon*		20	W
5	Allium	*Allium*	*sphaerocephalon*	*trachypus*	1	Gr
6	Allium	*Allium*	*amethystinum*		1	W
7	Allium	*Allium*	*bourgeaui*		1	Ae
8	Allium	*Allium*	*chamaespathum*		2	Gr
9	Allium	*Allium*	*dilatatum*		1	R
10	Allium	*Allium*	*commutatum*		2	W
11	Allium	*Allium*	*integerrimum*		2	Gr
12	Allium	*Allium*	*scorodoprasum*		6	W
13	Allium	*Allium*	*sphaerocephalon*	*aegaeum*	1	Ae
14	Allium	*Allium*	*proponticum*		1	Ae
15	Mollium	*Allium*	*pthioticum*		1	Gr
16	Mollium	*Allium*	*longanum*		1	W
17	Mollium	*Allium*	*trifoliatum*		1	W
18	Scorodon	*Allium*	*laganophyllum*		1	R
19	Codonoprasum	*Allium*	*flavium*		11	W
20	Codonoprasum	*Allium*	*subhirsutum*		1	Gr
21	Codonoprasum	*Allium*	*pallens*		5	W
22	Codonoprasum	*Allium*	*caristannum*		1	W
23	Codonoprasum	*Allium*	*paniculatum*		3	W
24	Codonoprasum	*Allium*	*tardans*		1	R
25	Codonoprasum	*Allium*	*staticiforme*		4	Ae
26	Codonoprasum	*Allium*	*euboicum*		3	R
27	Codonoprasum	*Allium*	*dendiferum*		3	W
28	Brevispatha	*Allium*	*callimischon*		1	W
29	Melanocrommyum	*Allium*	*cyrilli*		1	W
30		*Allium*	*autumnale*		1	Gr
31		*Allium*	*achaium*		1	En
Total no. of accessions					**164**	

* W = widespread; Gr = Greece only; R = regional; Ae = Aegean islands; En = endemic

Description of the CGN onion and leek collection

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The collection

The *Allium* collection originates from the former Institute for Horticultural Plant Breeding (IVT). This collection included mainly onion and leek cultivars obtained from Dutch seed firms and was collected in the framework of ECP/GR (van der Meer and van Bennekom 1983). Much attention was paid to rationalizing the collection by means of bulking duplicates, both from onion and leek. This effort resulted in a considerable reduction of the collection (Boukema and de Groot 1991). The collection was replenished with material collected in a multicrop expedition in Pakistan (Hasmi *et al.* 1981), material collected in Bulgaria, accessions from multicrop expeditions funded by IPGRI in Egypt, from collecting missions in the framework of the German-Dutch cooperation in plant genetic resources to Turkey, Russian Federation (Daghestan), Armenia and Georgia, and from a multicrop collecting expedition to Uzbekistan (van Soest *et al.* 1998). The collection was completed with missing prominent open-pollinated Dutch and European varieties. Wild material was also introduced from botanical gardens and other genebanks. In the near future wild material collected in Uzbekistan will be added.

CGN actively takes part in the ECP/GR Working Group on *Allium* (Maggioni *et al.* 1999). The passport data of the CGN *Allium* collection are included in the European *Allium* Database (EADB) maintained by Horticulture Research International, UK. CGN gives high priority to the *Allium* collection and aims to create a representative sample of the total genetic diversity in onion and leek.

The collection presently includes 319 accessions of seed-propagated *Allium*. The species and cultivar groups included in the *Allium* collection are presented in Table 1. The population types of the cultivated material are given in Table 2. The kurrats are landraces originating from Egypt. The leeks are predominantly cultivars from the Netherlands, but cultivars from France and Denmark and some landraces from Bulgaria are also included. The onions are mostly varieties from the Netherlands and Japan, but the collection also includes landraces from Bulgaria, Egypt, Pakistan, Russian Federation and Uzbekistan. The bunching onions are nearly all varieties from Japan. The Chinese chives are landraces from Australia and Thailand. The population type of 37 accessions of the cultivated material is not known.

Regeneration

About 150 accessions will be added to the collection after they have been regenerated. This includes approximately 25% onions. Difficulties arise in the regeneration, particularly for some of the short-day material, e.g. accessions from Pakistan. Regeneration of wild material also often causes problems. It can take several years before enough seed is produced to fulfil the CGN standards.

The number of plants used for regeneration is 60-120. After onion bulbs have been harvested, dried and potted, they are placed in a non-heated glasshouse for overwintering. Due to problems with *Fusarium* attack, leek seedlings are directly planted in pots and overwintered from November onward in a glasshouse at 5-10°C. As soon as flowers appear, the plants are transferred to isolation rooms and pollinated by blowflies.

Table 1. The *Allium* collection per species and cultivar group

Species/Group	No. of accessions
Cultivated	
A. ampeloprasum group kurrat	11
A. ampeloprasum group leek	71
A. cepa group dry bulb onion	157
A. cepa group silverskin onion	6
A. cepa group spring onion	2
A. cepa group unknown	1
A. fistulosum, japanese bunching onion	32
A. cepa x *fistulosum*, japanese bunching onion	1
A. tuberosum, chinese chive	5
Wild	
Subgenus *Allium*	
A. ampeloprasum	7
A. flavum	1
A. guttatum	1
A. sphaerocephalon	3
Subgenus *Rhiziridum*	
A. altaicum	5
A. drobovii	1
A. galanthum	4
A. oschaninii	1
A. pskemense	2
A. roylei	1
A. senescens	1
A. vavilovii	3
A. vavilovii x *cepa*	2
Other species	
A. schergianum	1
Total	**319**

Table 2. Number of accessions of the cultivated alliums per population type

Group	Population type*			Total
	L	B	U	
A. ampeloprasum group kurrat	11			11
A. ampeloprasum group leek	5	63	3	71
A. cepa group dry bulb onion	36	103	18	157
A. cepa group silverskin onion	2	4		6
A. cepa group spring onion		2		2
A. cepa group unknown			1	1
A. fistulosum, japanese bunching onion	2	16	14	32
A. cepa x *fistulosum*, japanese bunching onion		1		1
A. tuberosum, chinese chive	4		1	5
Total	**60**	**189**	**37**	**286**

* L = landrace; B = cultivar; U = unknown

Characterization and evaluation

Most of the onion and leek material (including kurrat) has been characterized respectively for 20 and 15 different traits, according to CGN descriptor lists (partly derived from UPOV and ECP/GR descriptor lists). Characterization for the minimum descriptors, as agreed at the fifth meeting of the ECP/GR Working Group on *Allium* in 1995 (Gass *et al.* 1996) and in the EU project GEN RES CT95-20, has started. Cultivars are characterized during bulb/mature plant production for regeneration.

Evaluation data on characters such as diseases are obtained from users of the material. Data on resistance to *Peronospora destructor*, *Sclerotinium cepiforum*, *Botritis aclada* and *B. squamosa* are available. Data on *Thrips tabaci* will become available in the near future.

The characterization and evaluation data can be found on CGN's Web site (<http://www.plant.wageningen-ur.nl/cgn/>).

Seed exchange

Packages with about 300 seeds per accession can be made available upon request.

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The Allium *collections at Plant Research International, with special reference to the vegetatively maintained leek (von Bothmer) collection*

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Introduction

Since 2000 Plant Research International (Plant RI) is a private research company under the umbrella of DLO (Agricultural Research Service). Together with Wageningen University, Plant RI forms the Wageningen University and Research Centre (Wageningen-UR). Research at Plant RI is carried out from the ecosystem to the gene levels. The *Allium* research group of Plant RI is part of the business unit (BU) Genetics and Breeding. The group consists of 5-10 researchers and the main research themes of the group are advanced breeding methods (marker-assisted breeding, genetic transformation and molecular cytogenetics), resistance (biotic and abiotic stress) and quality (sulphur and carbohydrate metabolism). More information about the group can be found at <http://www.plant.wageningen-ur/expertise/alliumresearch>.

Plant RI *Allium* collections

The availability of well-characterized collections is generally considered of significant importance for a successful crop breeding and genetics programme. The *Allium* collection within the Genetics and Breeding Business Unit at Plant RI consists of an onion collection, a leek and garlic collection and an ornamental *Allium* collection. The above-mentioned *Allium* collections are not freely accessible. However the Centre for Genetic Resources (CGN) of Plant RI has a large and freely accessible *Allium* collection (<http://www.plant.wageningen-ur.nl/cgn/>). Therefore there are two large *Allium* collections at Plant RI: the freely accessible collection of CGN and the private collection of the BU Genetics and Breeding.

The onion collection of the BU Genetics and Breeding consists of onion and its wild relatives, interspecific hybrids, advanced breeding populations and transgenics. Most of the collection is maintained by seed, however part of it is vegetatively propagated. The ornamental *Allium* collection is rather small and comprises a number of species from the subgenus *Melanocromyum*. The leek and garlic collection is maintained for a large part vegetatively. The garlic collection is *in status nascendi* and presently comprises some 300 accessions that have not been well characterized. The Plant RI leek collection is subdivided into the so-called "von Bothmer collection", consisting of species from the *ampeloprasum* complex, and the leek and wild relatives collection.

The Plant RI von Bothmer collection

The von Bothmer collection is a unique collection acquired in 1982 by Q.P. van der Meer from R. von Bothmer, who collected this material mainly from the Greek Islands (von Bothmer 1974). The collection consists of species from the so-called *ampeloprasum* complex, namely *A. ampeloprasum*, *A. commutatum* and *A. bourgeaui* (Table 1). It is generally believed that the progenitors of the currently cultivated leek and kurrat can be found among the species of the *ampeloprasum* complex.

Table 1. The vegetative von Bothmer collection at Plant RI

Species	No. of accessions acquired in 1982 by Q.P. van der Meer from R. von Bothmer	No. of accessions present in 2001*
A. ampeloprasum	21	10/16
A. commutatum	39	20/55
A. bourgeaui	16	1/1
A. porrum	1	1/1
Total	**77**	**32/73**

* X/Y = number of so-called B-numbers/number of genetically different clones as identified via RAPD markers.

As can be seen from Table 1 a large number of *"B-numbers"* (sites of collection = accession) have been lost during the 19 years of vegetative maintenance. This is especially true for *A. bourgeaui*. In 1995 the complete von Bothmer collection was genetically fingerprinted using RAPD markers. Out of 42 tested Operon primer sets, five amplified a high number of PCR fragments and these were used to assess the genetic variation present in the collection. The Operon primer sets used were *V-04, B-19, E-11, E-17* and *X-09*. A number of accessions proved to be genetically uniform, indicating that only one clone was originally sampled (Table 2).

Table 2. Number of variable and non-variable accessions per *Allium* species in the von Bothmer collection

Species	Total no. of accessions	No. of variable accessions	No. of non-variable accessions
A. ampeloprasum	10	3	7
A. commutatum	20	15	5
A. porrum	1	0	1
A. bourgeaui	1	0	1

In some cases considerable redundancy was observed: for example one *A. commutatum* clone was present with 844 plants in the collection. Other accessions were polymorphic, indicating that genetically different clones were originally sampled from the same location (Table 2). A number of accessions are generatively and vegetatively maintained but the majority of the accessions are maintained vegetatively. Generally 5-10 plants per clone within an accession are vegetatively propagated. In 1995, before the RAPD screening was made, the von Bothmer collection consisted of 2165 plants; after the screening, this number was reduced to 571 plants, still maintaining most of the genetic variation present in the collection.

Kik *et al*. (1997) showed the potential value of this collection for the introduction in leek of an F1 hybrid breeding system based on cytoplasmic male sterility (CMS). In the cultivated leek Kik and co-workers found only two mitochondrial (mtDNA) variants, whereas in the von Bothmer collection a considerable amount of variation was found, pointing at the increased chance of finding alloplasmic male sterility sources. Also Kik *et al*. (1997) showed that the species included in the von Bothmer collection could be succesfully crossed with leek. This means that the exploitation of this important gene reservoir for breeding new leek cultivars is possible.

The Plant RI leek and wild relatives collection

The leek and wild relatives collection consists of approximately 100 accessions acquired from public genebanks and through collecting missions (Table 3). The collection has been used in recent years for a PhD study on the genetics of resistance to white tip disease (*Phytophthora porri*) in leek (Smilde 1996) and also in a study regarding the introduction of resistance to thrips (*Thrips tabaci*) in leek. Both studies led to the distribution to plant

breeding companies of plant material with a high partial resistance to *Phytophthora porri* (Smilde *et al*. 1997) or to *Thrips tabaci*.

Table 3. The vegetative Plant RI leek and wild relatives collection

Species	No. of accessions
ampeloprasum group	**78**
A. ampeloprasum	53
A. pyrenaicum	2
A. porrum	1
A. commutatum	8
A. polyanthum	3
A. atroviolaceum	7
A. acutiflorum	1
A. macrochaetum	1
A. bourgeaui	2
sphaerocephalon group	**1**
A. proponticum	1
margaritaceum group	**1**
A. vineale	1
rotundum group	**15**
A. rotundum	1
A. dregeanum	1
A. scorodoprasum	13
Total	**95**

Acknowledgements

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Collections of vegetatively propagated onions in the Nordic Countries

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The Nordic Gene Bank (NGB) is a joint plant germplasm centre for the Nordic Countries (Denmark, Finland, Iceland, Norway and Sweden). The region covered extends between latitudes 54°N and 72°N and longitudes 24°W to 31°30'E where extreme growing conditions have developed highly adapted plants. NGB was established 22 years ago, which is a short time from a plant genetic resources perspective. However, it has successfully collected a large number of accessions of vegetatively propagated onions. A possible reason for this is that onions are often grown by smallholder farmers or in home gardens away from the cities. Farmers in the countryside gardens follow a strong tradition of replanting their own vegetatively propagated material. Therefore a large number of clones have been conserved, and gradually more are being discovered and added to the list.

The Nordic Countries distinguish between two types of vegetatively propagated onions: shallots (*Allium cepa* var. *ascalonicum* Backer) and potato onions (*Allium cepa* var. *solaninum* Alef). Traditionally, both varieties have been cultivated and used in the Nordic region. A 1920 textbook indicates: "*The shallots are more pungent and used as spice, while the potato onions are milder and consumed as vegetable*" (Lundèn 1920).

Botanically, the two varieties, both vegetatively propagated, are distinguished as follows (Hanelt 1990; Gladis 1996):

- **Shallot:** more or less bowl-shaped, mostly small bulbs, which frequently break through the skin of the mother bulbs;
- **Potato onion:** with fewer round, somewhat flattened larger bulbs, which are enveloped in a common tunica, or break it later than the former.

Potato onions were introduced into Finland from Russia during the latter part of the 19th century by Russian monks, and possibly distributed further into the Scandinavian Peninsula. Formerly, they were known to be widely cultivated in Finland, Estonia, Latvia, Lithuania, Poland and Sweden.

Shallots are thought to have been introduced into the Nordic Countries from Central Europe.

Collecting activities

In Finland, 121 samples of vegetatively propagated onions, mostly potato onions, were collected (1984-1989). This material has been characterized and the collection presently comprises approximately 50 accessions. Among these, 10 accessions have been selected as core accessions.

In Norway, 17 accessions of shallots have been collected. In Sweden, the Seed Saver Organization SESAM collected 6 accessions of potato onions and this material is stored on-farm by the members.

In Denmark, recent collecting of shallots resulted in 23 samples obtained from 23 different localities. During the last 10-20 years the commercial production and maintenance of shallots has diminished to an insignificant level, therefore the consumption of shallots is covered by home production and maintenance, or by import. Up to now the shallots have been preserved in an "in-garden" conservation system. Many of the accessions collected may probably be considered as landraces or local varieties, of value not only as plant genetic resources but also for their historical importance.

Maintenance of the collections

The conservation of the collected material is a national responsibility and thus the material is kept in clonal archives in each country (Table 1), while NGB is responsible for documentation and information systems related to the collections. As the maintenance of the vegetatively propagated onions is very labour-demanding and they are susceptible to diseases and sensitive to frost, this material is classified as endangered germplasm. Therefore, the NGB Board decided to establish an *in vitro* base collection in the genebank. Presently, this collection is maintained at Balsgaard Research Station, Swedish Agricultural University, but it will be transferred to the NGB in the near future. The information on all the material, however, is maintained by the NGB. . Material of the Finnish and Norwegian collections are safety-stored in the Vegetable Research Institute in Olomouc, Czech Republic (VRIO).

Table 1. *Allium* material conserved in the Nordic countries—characterization and safety-duplication status

Country	No. of accessions	*In vitro*	Passport data	Characterization data	Safety-duplication
Denmark	23	1	0	0	1 NGB
Finland	132 (50)	10	50	50	53 VRIO, 10 NGB
Norway	17	17	17	17	17 VRIO, 17 NGB
Sweden	6	6	0	0	6 NGB, on-farm
Iceland	0	0	0	0	0

The Finnish and Swedish materials are mainly potato onions, while the accessions in the Norwegian and Danish collections are predominantly shallots.

The collections from Finland and Norway have been extensively characterized: 132 Finnish accessions are identified by 35 passport and 38 characterization and evaluation descriptors taken from the IPGRI list, while the Norwegian material is characterized with 12 passport and 14 morphological descriptors, using NGB and UPOV characters.

The Danish and the Swedish material is presently being collected and characterized. In Iceland this kind of onion is not cultivated.

It is our aim to secure safety-duplication by growing the material in the existing national clonal archives and centrally maintain an *in vitro* base collection in the Nordic Gene Bank. Alternatively, the material stored *in vitro* may be kept at two separate locations.

Recommendations for the continued work with vegetatively propagated onions in the Nordic-Baltic region are:

- Further collecting in the region
- Rationalization of collections by identification of duplicates using morphological and molecular markers; continued work may comprise evolutionary and taxonomic aspects
- Investigation of factors affecting clove size and flowering
- Evaluation for disease resistance among the germplasm.

A multidisciplinary Swedish project proposal on potato onions is presently under preparation.

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Vegetatively propagated Allium *genetic resources in Poland*

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The *Allium* germplasm collection at the Research Institute of Vegetable Crops (RIVC) in Skierniewice includes 1322 accessions of cultivated and wild *Allium* species. The current status of *Allium* germplasm is given in Table 1. Passport data for all collected accessions were standardized in accordance with the FAO/IPGRI Multicrop Passport Descriptors and FAO-WIEWS Descriptors.

In the frame of the national vegetable germplasm conservation programme, three field collections of vegetatively propagated *Allium* species are maintained in two regions of Poland. In 2001 the field collections include garlic, shallot and edible and wild species.

Garlic collection (*A. sativum* L.)

The garlic collection established in 1986 is located in Krzczonów in southern Poland (300 km south of Skierniewice), in the old centre of garlic cultivation. In 2001 the garlic collection consists of 275 accessions (134 accessions for winter cultivation and 141 for spring cultivation) (Table 1). Part of the material is maintained in field collection after 3-year trials; other accessions are under multiplication and preliminary evaluation.

Table 1. Status of *Allium* germplasm, Skierniewice, 2001

Species	Total no. of records in database	Passport data	Evaluation / characterization	Seed in long-term storage	Field collection 2001	Duplication
A. cepa	248	248	62	248		57
A. cepa var. *aggregatum*	160	160	129	34	139	10
A. sativum	495	495	436		275	78
A. porrum	21	21		21		
Other *Allium*	398	398	131	81	240	
Total	**1322**	**1322**	**758**	**384**	**654**	**145**

As shown in Table 1, the total number of garlic accessions in the field in previous years was 495, but during a few unfavourable winters (especially in 1996) part of the very valuable material was totally frozen or partly damaged. The most dangerous factors during the winter months are frost, lack of snow and wind.

To minimize the loss of germplasm, each year part of the bulbils and bulbs is kept for planting during the following season and, in case of heavy losses in the field during winter, these materials are planted in spring to regenerate lost material. Additionally, the field containing garlic is covered with straw during winter to protect it against frost and wind. In spite of this protection, it was not possible to keep all materials for very long. These kinds of protections have advantages and disadvantages and the result depends on the weather conditions. Some of the accessions were regenerated by using duplicates obtained from other collections (Olomouc, Czech Republic; Moscow, Russian Federation; and Madison, USA).

It seems to be safe to duplicate material in other places or, on a larger scale, to use duplicates maintained as *in vitro* cultures or cryopreserved.

After multiplication, garlic accessions are included in 3-year trials (3-4 replications) to evaluate the economic value. After a 3-year research cycle, accessions are maintained in a

field collection in one replication (100 plants for each accession). Evaluation is conducted according to the descriptors elaborated by IPGRI and RIVC.

Accessions maintained in the collection are documented with passport data. Minimum characterization of 14 traits has been carried out for 259 garlic accessions and sent to the European *Allium* Database (EADB).

Evaluation data were scored for 436 accessions, according to IPGRI standards and the needs of the breeders. Evaluation data include 31 characters from the IPGRI descriptor list, 39 characters important for the economic value of garlic, estimation of variability of six enzyme systems, content of alliine, dry matter, sugars and vitamin C. Garlic accessions were divided into similar groups on the basis of bulb structure.

Computerized documentation is available for 260 accessions and covers bulbs, cloves, flower stalk and a cross-section of the heads. Part of the material (about 40%) is also documented with slides, photographs and video films, which record the original habitat of the collected accessions.

On the basis of the results obtained, several garlic accessions have been selected for breeding programmes and 3 new garlic cultivars ('Harna', 'Orlik' and 'Arkus') were registered.

Cryopreservation of garlic germplasm

Research work on cryopreservation of garlic germplasm is continuing after the investigations started with an IPGRI-funded project carried out in collaboration with IPK (Makowska *et al.* 1999). The aim of the study is to establish effective methods for garlic germplasm micropropagation and long-term conservation using *in vitro* culture and liquid nitrogen.

This investigation has explored the use of vitrification methods applied to the bulbils of 18 garlic accessions (*A. sativum* L.) from the Polish collection. The results showed that the best kind of explants to secure high regrowth frequency is shoot tips isolated from bulbils. To choose the optimal medium for micropropagation and regeneration six variants of MS medium (Murashige and Skoog 1962) (MS 0 to MS V) were used. The best medium for micropropagation was MS medium variant I (MS I). The best medium for regeneration was MS IV. The vitrification test was most successful when meristems about 1 mm in diameter and 3 mm in length were used with MS I. Thirty-one vitrification experiments were carried out on the 18 garlic accessions.

The survival and regrowth of all control shoot apices after treatment with vitrification solution (PVS 3) was relatively high for each accession (up to 100%). The best survival and regrowth of cryopreserved apices of garlic accessions after 1 month and 3 months were obtained when samples treated with vitrification solution PVS 3 were immediately immersed in liquid nitrogen (70-100%).

Relatively high frequency of shoot formation (70-80%) was obtained with 2 accessions (230 and 354) maintained for 1 month and for 3 months in liquid nitrogen, after 60-90 minutes of incubation of the apices with vitrification solution PVS 3 solution.

In the case of accession 242 stored for 1 month in liquid nitrogen, a 50% regrowth rate was obtained after 150 min of incubation of apices in vitrification solution PVS 3 and 60% after 90 min of incubation of apices stored in liquid nitrogen for 3 months.

The plants of the 18 garlic accessions used in vitrification tests were planted in field trials after successful adaptation in the greenhouse.

The results showed that survival and regrowth after cryopreservation were dependent on genotype and size of the bulbils. Further experiments should be performed to optimize the cryopreservation protocol. Other factors such as the duration of the treatment with vitrification solution PVS 3 and the period of cooling of apices in liquid nitrogen should be investigated (Makowska *et al.* 1999; Makowska and Kotlińska 2000, 2001a, 2001b, 2001c).

Shallot collection

The collection of shallot landraces was established at RIVC in 1988, based on landraces originating from Poland and neighbouring areas. There are no advanced cultivars of shallot in Poland, since only landraces are grown in home gardens.

Currently, 160 accessions are recorded in the database, but 139 accessions are maintained in the field collection. These are of Polish origin, except for 35 accessions originating from Albania, Moldova, Russian Federation, Slovakia, Ukraine and the USA. Additionally, 34 seed samples are deposited in seed storage. All collected accessions of shallot are documented with passport data and have been evaluated for 40 traits, according to IPGRI, USDA and UPOV descriptor lists, including susceptibility to onion fly and viral diseases. Visual digital documentation is available for 40 accessions and includes bulbs, clusters, cross-section of bulbs, etc.

In 10 landraces of shallot (*Allium cepa* L. var. *aggregatum*), the level and changes of flavonol content (kaempferol, myrecetin, quercetine) were investigated during the vegetation period, during storage and in desiccated shallot (Horbowicz and Kotlińska 1998, 2000, 2001a, 2001b).

Edible and wild species collection

The collection of edible and wild species maintained in Skierniewice includes 240 accessions originally collected in Central Asia and Siberia and wild species occurring in Poland. This collection includes species that can only be propagated vegetatively and species that can be more easily vegetatively propagated than by using seeds. Nearly all accessions are documented with passport data and are included into the EADB. Characterization and evaluation, according to IPGRI and USDA descriptor lists, were carried out for 131 accessions and cover 49 traits. The evaluation data were entered into a computerized database. Sixteen accessions of *Allium fistulosum* were investigated for their adaptation to Polish conditions (Kotlińska and Kojima 2000). The distribution of wild *Allium* species growing in wild habitats and their role as weeds of cultivated crops were evaluated (Kotlińska 1999).

Flavonol content (myrecetin, kaemferol, quercitine) was determined in the leaves and bulbs of nine wild *Allium* species: *A. ledebourianum, A. galanthum, A. altaicum, A. ampeloprasum, A. caesium, A. proliferum, A. fistulosum, A. nutans* and *A. vavilovii* (Horbowicz and Kotlińska 1998, 2000).

Onion collection

The germplasm collection of *A. cepa* consists of 248 accessions. The onion collection in Skierniewice is periodically planted in the field for characterization, evaluation and seed increases, when newly collected accessions are introduced or regeneration is necessary. Minimum characterization (14 traits) of 62 onion accessions from the genebank were scored and included into the EADB. Characterization covers 46 morphological and economical traits, following the descriptor lists developed by IPGRI, and partly established by UPOV and RIVC. Flavonol content (keampferol, myrecetin, quercitine) was also analyzed in 20 onion cultivars (Horbowicz and Kotlińska 2000).

Collecting missions

Collecting missions in different regions of Poland and neighbouring countries are organized every year to collect and protect indigenous germplasm threatened by extinction. When possible, the Polish Gene Bank joins in explorations organized by other organizations such as the EKO Foundation, IPGRI, USDA, the Vavilov Institute, etc. Between 1997 and 2000 the Polish Gene Bank organized or participated in a total of 26 expeditions during which 2388 accessions of vegetable crops and relative wild species were collected, including

367 accessions of *Allium*: 72 accessions of onion, 80 of shallot, 138 of garlic, 7 of leek and 70 of other *Allium* species (Table 2). Each collected seed sample was split into two parts: one part is added to the base collection, while the other is used for multiplication and preliminary evaluation.

Table 2. Vegetable germplasm collected during explorations from 1997 to 2000

Date	Area	Country	Total no. of accessions collected	No. of species	No. of *Allium* accessions				
					Onion	Shallot	Garlic	Leek	Other
Sept 1997	Javorniky, Horna Orava	Slovakia	63	9	3	2	7		
Sept 1997	Lwow region	Ukraine	172	17	7	5	15		1
Sept 1997	Zarnovica, Banska Stiavnica	Slovakia	20	6	1		9		
Oct 1997	Bielsko Biala, Zywiec	Poland	68	15	2	3	9	2	1
Nov 1997	Zamosc	Poland	115	25	6	3	1	1	
Oct 1997	Wielkopolska	Poland	9	4					
Aug-Sept 1998		Ukraine/ Moldova	332	37	12	7	19		4
Oct 1998	Biala-Podlaska	Poland	146	25	6	1	8	1	2
Oct 1998	Bialystok	Poland	111	21	1	10	7		1
Oct 1998	Zamosc	Poland	143	20	5	4	6	1	
Nov 1998	Pieniny-Bieszczady	Poland	58	7					
Nov 1998	Zielona Góra	Poland	33	9		2			
Nov 1998	Lomza	Poland	7	1		4			
May 1999		Romania	16	13					
Sept 1999	Krym	Ukraine	4	4			1		3
July 1999	central-eastern region	Poland	145	30	3	3	3		1
July 1999		Syria	115	39	3		1		15
Aug 1999		Turkey	139	17					9
Aug 1999		Greece	148	35	1		1	1	24
Sept 1999	Beskidy	Poland, Czech Rep, Slovakia	18	12			3		2
Sept 1999	Zakarpacie	Ukraine	99	21	5	1	14		
Oct 1999	Kielce	Poland	27	4	1	1	6		
Oct 1999	Podkarpacie	Poland	73	16	2	4	7	1	
Oct 1999	Narew	Poland	63	12	6	7	5		1
Oct 2000	Tarnow	Poland	114	24	7	2	6		4
Oct 2000	Sokolka	Poland	150	28	1	21	10		2
	Total		**2388**		**72**	**80**	**138**	**7**	**70**
					Total *Allium* accessions = 367				

In selected areas, each village was explored to collect not only seed material, but also all available information about local growing systems, local methods of plant protection, type of use (food / medicinal plant), etc. Coordinates of the collecting sites were recorded with GPS (Global Positioning System). This information is also a very useful tool for choosing proper places for *in situ* and on-farm conservation. The sources of collected material were mostly local markets, home gardens and home storages in isolated villages, where aged farmers still maintain local cultivars of various vegetables in small quantities for domestic use. Wild species were collected from their natural habitats.

Utilization and perspectives

From 1997 to 2000 new introductions into the genebank consisted of 243 accessions of *Allium* germplasm from Polish donors, 367 accessions from explorations and 58 accessions from abroad.

Between 1997 and 2000, 336 seed samples of *Allium* were distributed to users in Poland, including 124 accessions of onion, 50 of shallot, 135 of garlic, 7 of leek and 20 of other *Allium* species. Since 1997, 173 seed samples have been sent to users abroad, including 62 accessions of onion, 1 of leek, 5 of shallot, 21 of garlic and 84 of other *Allium* species.

The most requested materials are those that provide new sources of resistance to diseases and pests and environmental stress tolerance. More often, breeders prefer to find sources of various economic traits in the existing cultivars rather than in wild or primitive populations. However, landraces originated from Central Asia and Siberia are used as a source of high dry matter content and also for the quality of dry skin and as a source of traits for sterility, earliness, good storability and adaptation to different environmental conditions.

Many *Allium* accessions have been used in research programmes at research institutes and universities. Research on *Allium* genetic resources is directed towards improving the availability and utilization of useful germplasm. Further efforts are ongoing to:

- improve germplasm characterization and evaluation using modern methods on a larger scale;
- continue investigation on chemical, biochemical and nutritive composition of selected *Allium* species, which could be introduced as new crops for consumption and as ornamentals;
- use *in vitro* culture and cryopreservation methods to maintain duplicates of vegetatively propagated garlic species;
- increase the number of explorations to collect, as far as possible, native landraces still existing in Poland;
- undertake taxonomic identification of uncertain species of *Allium;*
- eliminate viruses in garlic and shallot collections;
- organize on-farm conservation in selected regions of Poland to maintain and reintroduce some valuable vegetable landraces;
- increase the number of accessions safety-duplicated in other genebanks;
- broaden collaboration with other networks, institutions and non-governmental organizations within the country and abroad;
- search for additional sources of funding to allow further activity, mainly collecting missions and multiplication of newly introduced germplasm. Unstable and insufficient funds make it impossible to plan future work and to ensure the conservation of existing germplasm.

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Status of vegetatively propagated Allium *collections in Portugal*

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The Banco Português de Germoplasma Vegetal (BPGV) began to establish the Portuguese field collection of vegetatively propagated *Allium* in 1994. This work is still being continued.

Collecting missions have been carried out across Portugal. During these missions it has been observed that, particularly in the East of the country, local *Allium* populations are in danger of disappearing, owing to the fact that low-priced new cultivars of garlic produced across the international border have been flooding into Portugal. However, many Portuguese farmers continue to cultivate the traditional populations (known as *"alho da matança"*) because they believe it tastes better than the imported cultivars and is better for sausage preparation.

Responsibility for maintenance of the collections lies with the national government.

The Portuguese collection of vegetatively propagated *Allium* includes both wild and cultivated material (Table 1). The cultivated materials are maintained as field collections in different regions of the country, while the wild materials are kept at BPGV as bunches in pots, in a greenhouse protected against the weather.

Table 1. Species of the Portuguese *Allium* collections

Species	No. of accessions
A. ampeloprasum L. (landrace)	14
A. *ampeloprasum* L. (wild)	11
A. *baeticum* Boiss.	1
A. *cepa* L. Aggregatum group (landrace)	6
A. *roseum* L.	5
A. *sativum* L. (landrace)	292
A. schoenoprasum L.	2
A. scorzonerifolium Desf.	1
A. *sphaerocephalon* L.	2
A. *vineale* L.	2
Total	**336**

Collecting missions

Some accessions were lost over the years due to pest attacks or diseases and to flooding caused by heavy rains in the winters of 1998 and 1999. Therefore it became necessary to re-collect the material in order to replace the lost material. This was done in 2000. Systematic collecting missions of wild *Allium* will continue to be carried out all over Portugal.

Evaluation, selection and breeding

Systematic morphological characterization has been carried out for all cultivated accessions of garlic, leek and shallots.

Molecular evaluation of the *A. sativum* collection has been in progress since 2001. In 1996, following a joint collecting mission with Prof. Takeomi Etoh, of the Kagoshima University, Japan, 14 accessions of the Portuguese collection were included into a research programme.

In order to obtain a Portuguese population, a breeding programme has been carried out by BPGV and ISA (Instituto Superior de Agronomia).

In vitro collection

BPGV has begun to establish an *in vitro* virus-free base collection.

Documentation

All information about the collections is recorded and a copy of this database has been provided to D. Astley, manager of the European *Allium* Database.

Safety-duplication

In 1995, 22 garlic accessions were sent to P. Havránek in Olomouc, Czech Republic, for safety-duplication, as recommended by the ECP / GR Working Group on *Allium*.

Status of the Spanish garlic collection

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In Spain, conservation, characterization, evaluation and collecting of *Allium* crops and wild species has been supported from the onset by INIA (Ministry of Agriculture). During the period 1 March 1996-28 February 2000, the work was supported by the EU through the GEN RES *Allium* Project. Currently this work is only funded by INIA. One of the objectives of this project is to produce virus-free cultivars in the Banco de Germoplasma, through the Department of Microbiology of the Faculty of Sciences, University of Córdoba (UCO). There are currently 45 virus-free clones and a further 10 cultivars are being cleaned.

Within the GEN RES *Allium* project a garlic "core collection" was developed by CIFA and IPK-Gatersleben, in which 25 accessions from the INIA genebank were included, as defined in the last report of the project. The option of increasing the number of cultivars with some of those virus-free clones, which show much better genetic variability, will be discussed in due course.

As part of the GEN RES project, the characterization of the garlic collection was also carried out and the data were forwarded to the Genetic Resources Unit of Horticulture Research International, Wellesbourne, UK. Two additional files were also forwarded, containing updated data for those accessions collected after the end of the GEN RES project.

The INIA germplasm bank is managed as follows:

- Preliminary soil analysis (in May) to check the presence of pathogens (*D. dipsaci* and *S. cepivorum*)
- Plot solarization during summer with transparent plastic (200 g)
- Application of insecticide to the soil at the time of fertilization before planting
- Seed disinfection after seed threshing
- Planting of all available cultivars
- Each cultivar is planted when the garlic cloves start to produce a small stalk and is approximately half of the clove length
- Preventive control of pest and diseases during production
- Hand weeding
- Harvesting is carried out several times, but only when each cultivar has ripened with the cloves well defined in the bulb and all except the last 2-4 leaves are wilted.

After harvesting, cloves are left in the open to dry for 3-4 days. After cleaning, plants are taken to the warehouse, where after 5-6 weeks the “bulbs” are completely cleaned by removing roots and leaves. During the whole process, in the field and in the warehouse, a continuous selection is performed discarding any bulb with wounds, rotten tissue, etc.

Regarding virus-free material, once the microbulbils are received from *in vitro* culture, they are planted in pots in soil free of pathogens and fertilized (slow-release fertilizer). Pots are placed in a structure protected with an insect-proof net and irrigated using drip irrigation. During the growth cycle, care is taken to prevent contamination by diseases and pests. After harvesting, a similar process is followed to that above, albeit in a different warehouse for this virus-free material, which is subsequently stored in a different building than the standard material. Details of the CIFA virus-cleaned material are given in Table 1 below.

Table 1. Garlic accessions cleaned from viruses at CIFA Córdoba (total: 45 virus-free accessions)

Accession number (ACC_NO)	Cleaned accessions		Starting date
	Name (CULT_NAME)	Code	
1	Cabeza del Obispo	CO	1997
5	106/87	NA	1997
10	T-240	NZ	1996
11	Zahorsky	Z	1997
12	T-530	NX	1996
19	485/87	NU	1996
23	1054/86	NB	1996
33	117/86	N	1997
35	1035	NE	1997
43	Bañolas	YA	1996
44	Chino rojo	OY	1996
48	Chinchón	CN	1997
51	Blanco de Ronda	W	1996
52	Blanco de Valladolid	BV	1997
72	R. Cuenca	R	1995
73	Saturnino	S	1997
79	Chino blanco	C	1997
81	Aja rondeña	XR	1996
82	Indio	IN	1996
100	Chinés	CX	1995
101	Huelma 3	HV	1996
104	Centenario	CT	1996
112	Huelma J	HJ	1997
115	Arica	AR	1996
134	Hinojosa	JJ	1995
137	Lorenzo	L	1997
142	Arzúa	AZ	1995
144	Ancud	AN	1996
146	Aja de Fuente Palmera	XF	1995
152	A. Alvarez	AA	1997
155	R. Gil	RG	1995
157	A. Campo	AC	1997
160	J. Moncayo Becerra	J	1996
215	Basic I (Mª Jesús)	B	1997
329	Colorados	CL	1997
2	Morado de Pedroñeras 1°	MP	1998
3	Morado de Córdoba	MC	1998
14	Taiwan	TA	1998
27	879/86 J	NJ	1998
54	Blanco de Vallelado	BL	1998
74	Gigante holandes	G	1998
85	Rojo de Rute	RR	1998
90	In Vitro	V	1998
95	Chino cidaco	CC	1998
159	Peñalsordo	PÑ	1998

Germplasm exchange of vegetative alliums—minimum phytosanitary requirements

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Introduction

In addition to the main task of a genebank, namely the maintenance and storage of germplasm, an important element of genebank work is also the exchange of plant material for research, crop improvement, plant breeding or conservation. The movement of genetic resources around the world involves the risk of dissemination of pathogens and pests (fungi, viruses, nematodes and mites) into other regions where they do not so far occur. Therefore, the increasing extent of germplasm exchange requires measures to ensure the phytosanitary safety of germplasm transfer at international level.

In cooperation with FAO and IPGRI, a series of crop-specific technical guidelines for the safe movement of germplasm has been published since 1989. The technical guidelines for *Allium* spp. were published in 1997 (Diekmann 1997). They had been developed at an FAO-sponsored meeting of experts in Prague in 1995. The guidelines make recommendations and describe procedures that minimize the risk of pest distribution, coupled with the exchange of allium germplasm.

Diseases in vegetative alliums

Usually, vegetatively propagated alliums are permanently maintained in the field. Depending on the local conditions, the plants remain on the same field plot for one year or longer. The IPK genebank runs a 5-year rotation cycle, i.e. the vegetative collections are replanted every 5 years onto a new field plot. In other countries, particularly under warm climatic conditions, the plants have to be replanted every year due to the very high pathogen incidence. In southern Europe, the collections are planted in autumn and harvested in spring. After a storage phase and selection for healthy material, the collections are replanted on new plots or areas that have been cleaned in the meantime by solarization or other methods.

The permanent field maintenance results in the accumulation of fungal and viral diseases in most of the species. Fungi and viruses can survive on leaves, bulbs, cloves and bulbils over a long period. These organs, which are a permanent source of new infections, are so far the only types of material used for germplasm exchange. Therefore, the danger of dissemination of pathogens and pests through the transport of infected material is very high.

Table 1 shows a selection of the main pathogens that may be transmitted with bulbs, cloves and bulbils. *Allium* is subject to fungal and viral diseases, as well as to nematodes and mites. On the leaves, downy mildew and botrytis leaf blight are observed almost every year under field conditions at IPK. The highest infection level of *Peronospora destructor* is found on *A.* x *proliferum* and *A. fistulosum*. The disease can be reduced, but not prevented, by spraying with fungicides. Also *Botrytis* spp., *Alternaria* spp., *Fusarium* spp. and *Sclerotinia* spp. are frequently observed mainly as secondary parasites on already infected material.

Table 1. Frequently occurring pathogens in vegetative alliums, which may be transmitted with germplasm (bulbs, bulbils, cloves and *in vitro* culture)

***Allium* species**	**Fungi**	**Viruses***
A. sativum (garlic)	*Alternaria porri* *Fusarium* spp. *Sclerotinia cepivorum*	OYDV, LYSV, GCLV, SLV, allexiviruses, GDV
A. ampeloprasum L. (great-headed garlic)	*Alternaria porri* *Cladosporium allii-cepae* *Sclerotinia cepivorum*	OYDV, LYSV, GCLV, SLV, allexiviruses
A. cepa var. *aggregatum* (shallot)	*Peronospora destructor* *Botrytis squamosa* *Botrytis allii* *Alternaria porri* *Cladosporium allii-cepae* *Fusarium* sp. *Sclerotinia cepivorum*	OYDV (only on material of European origin) SYSV (only on material of Asian origin) SLV allexiviruses
A. x *proliferum* (top onion)	*Peronospora destructor* *Botrytis allii* *Alternaria porri* *Sclerotinia cepivorum*	OYDV, LYSV, SLV
A. fistulosum (bunching onion)	*Peronospora destructor* *Botrytis allii* *Alternaria porri* *Sclerotinia cepivorum* *Cladosporium allii-cepae* *Fusarium* sp.	LYSV, SLV, allexiviruses
A. chinense	*Sclerotium cepivorum* *Fusarium* sp.	
Wild species	*Sclerotium cepivorum* *Cladosporium allii-cepae*	Differing according to species (LYSV, SLV, GCLV)

Source: based on Diekmann (1997) and Rabinowitch and Brewster (1990).
* Abbreviations: OYDV = onion yellow dwarf virus; LYSV = leek yellow stripe virus; GCLV = garlic common latent virus; SLV = shallot latent virus; SYSV = shallot yellow stripe virus; GDV = garlic dwarf reovirus; allexiviruses (garlic virus A, B, C, D, shallot virus X).

Likewise, virus infections are common in most of the *Allium* species. At the end of May, different symptoms of yellow stripes and mosaics caused by different viruses can be observed on the leaves of field-grown plants. The most frequent viruses are the potyviruses OYDV, LYSV, SYSV, the carlaviruses GCLV and SLV, and the allexiviruses, formerly called mite-borne filamentous viruses, which occur in many species.

In addition to the viral and fungal diseases, other pathogens are found in *Allium.* The stem and bulb nematode *Ditylenchus dipsaci* is widespread in cultivated alliums. More than 400 host plants have been described for *D. dipsaci.* Particularly garlic and shallot are affected. *Aceria tulipae* is also a serious pest of garlic but occurs in other *Allium* species as well. It is a vector of several viruses in the field and in storage (Diekmann 1997).

Virus screening tests

During the last few years virus screenings were performed on a large scale on the IPK garlic field collection and on a smaller scale on shallots, top onions, bunching onions and on the wild species collection (Tables 2 and 3).

Virus infections were found on leaves, bulbils and cloves in all the 105 tested accessions. GCLV and SLV were present in almost all accessions. GCLV was the most frequent virus with over 90% occurrence in both leaves and bulbils (Table 2). The virus infection in bulbils was somewhat higher than in leaves, perhaps caused by the later time of testing. Infections with more than one virus were prevalent.

The results of these observations and screening tests confirm the potential danger of disease dissemination through the exchange of vegetative material such as cloves and bulbils.

Table 2. Field infection of the garlic collection indexed in 1997 and 1998

Organ	No. of accessions	Virus-free accessions (%)	Infected accessions (%) with				
			OYDV	LYSV	GCLV	SLV	MbFV
Leaves (fresh)	105	0.02	50.5	34.3	93.3	77.1	27.6
Bulbils (stored)	87	0.00	70.0	55.0	95.4	81.6	27.6
Cloves (ripe)	27	0.00	96.3	40.7	96.3	55.5	40.7

Table 3. Field infection of shallots, top onions and wild species, detected with ELISA and TPIA (SYSV was not tested)

Allium species	Origin	Viruses in	
		Leaves	Cloves
Shallots			
All 298	Germany	SLV	OYDV, SLV, allexiviruses
All 591	Germany	LYSV, SLV, allexiviruses	OYDV
All 593	Poland	LYSV, SLV, allexiviruses	OYDV
All 596	Germany	OYDV, SLV	OYDV, SLV, allexiviruses
All 601	Germany	OYDV, LYSV, SLV, allexiviruses	OYDV
All 603	Germany	OYDV, LYSV, SLV, allexiviruses	OYDV
All 609	Germany	SLV, allexiviruses	OYDV, SLV
All 613	Germany	OYDV, SLV, allexiviruses	OYDV
All 614	Germany	SLV	OYDV, SLV, allexiviruses
All 617	Germany	OYDV	OYDV, SLV, allexiviruses
All 622	Germany	OYDV, SLV, allexiviruses	OYDV, SLV
All 718	Georgia	SLV, allexiviruses	SLV, allexiviruses
All 723	Canada	LYSV, SLV, allexiviruses	OYDV, LYSV, SLV, allexiviruses
All 726	Georgia	allexiviruses	OYDV, SLV
All 727	Georgia	LYSV, SLV, allexiviruses	allexiviruses
Top onions			
All 328	Germany	LYSV	non-tested
All 337	Germany	OYDV, LYSV, SLV	non-tested
All 342	Germany	OYDV, LYSV, SLV	non-tested
All 418	Germany	OYDV, LYSV, SLV	non-tested
All 1364	Russia	OYDV, LYSV, SLV	non-tested
All 1365	Russia	OYDV, LYSV, SLV	non-tested
Wild species			
A. albidum		LYSV	non-tested
A. angulosum		LYSV	non-tested
A. globosum		SLV	non-tested
A. hymenorrhizum		SLV	non-tested
A. carolineanum		LYSV	non-tested
A. obliquum		LYSV	non-tested
A. lineare, A. rubens, A. saxatile, A. senescens, A. nutans			No viruses detected

In vitro *cultures—a safe material for germplasm transfer*

In the technical guidelines for the safe movement of germplasm for *Allium* spp., some general recommendations and a decision key are given for the use of the safest mode of movement possible.

The safest method for *Allium* germplasm transfer is the movement of seeds, but this is not possible in vegetative alliums. For this material, the exchange of *in vitro* material is more suitable. *In vitro* plants are free of fungal and bacterial infections. Moreover, the

establishment of *in vitro* cultures via meristem cultures offers the chance to produce virus-free clones.

The EU Project GEN RES CT95-20, which ran from 1996 until 2000, enabled the IPK *In vitro* Group to establish a virus-free garlic collection. The results achieved are presented below.

Meristem culture

In vitro cultures may be obtained from meristems of different sources. We used ripe bulbils and unripe bulbs for their establishment (Fig. 1)

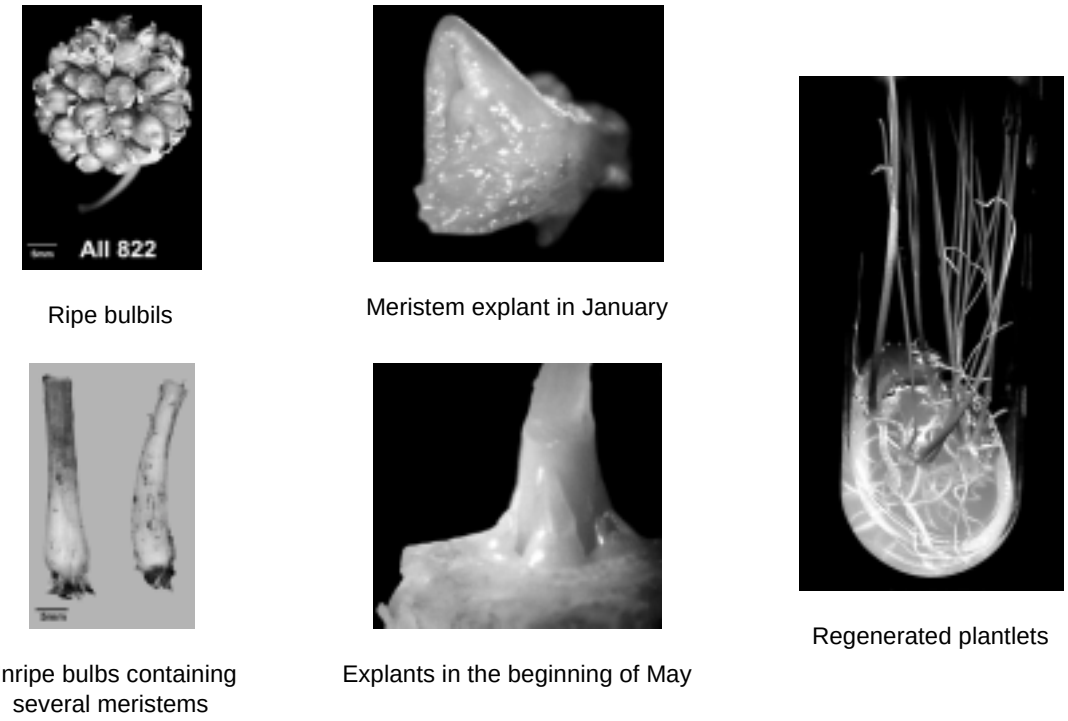

Fig. 1. Meristem culture from different explants.

The advantages of bulbils are their availability in great number and their storability for 8 months, thus resulting in an easier handling. Their disadvantage is that only one meristem per bulbil is available and, therefore, the labour input per meristem is relatively high. Unripe cloves were harvested in May at a phase of fast-growing bulbs. In this stage, the bulb contained several meristems with one or two leaf primordia. The disadvantage of this stage is the short time span of availability.

The meristems were cultivated on MS medium (Murashige and Skoog 1962) or BDS medium (Dunstan and Short 1977) with different hormones for the first 6 weeks of culture. The influence of the different culture media on plantlet regeneration was low compared to the influence of explant size and genotype. However, ribavirin, a virus-inhibiting chemical (50 or 100 mg/L) reduced the plant regeneration independently from its effect on viruses. It was possible to establish *in vitro* cultures of all garlic accessions tested so far.

Virus indexing

Virus indexing has been performed using ELISA (DAS, TAS, PTA), tissue print immunosorbent assay (TPIA) and electron-immunomicroscopy (performed in the Federal Biological Research Centre for Agriculture and Forestry (BBA), at Braunschweig). The ELISA technique with monoclonal and polyclonal antibodies, provided by BBA, was the

main indexing method. TPIA proved to be a suitable method for a quick screening of large quantities of accessions using cloves and bulbils. The plant material was indexed for OYDV, LYSV, GCLV, SLV and allexiviruses. The tests were performed several times. Material taken from *in vitro* cultures as well as *in vivo* conditions has been used.

Results

The meristem culture without chemotherapy resulted in average percentages of virus-free plants of 35% in 1997 and 25% in 1998. OYDV and LYSV could be eradicated up to 90%. GCLV was the most persistent virus with 38-60% occurrence in plants derived from meristem culture (Fig. 2). Ribavirin in the induction medium (50 mg/L) clearly helped the elimination of the tested viruses. In a comparative experiment on 39 accessions, ribavirin increased the number of virus-free plants from 25% in the controls up to 55% of all tested plants. GCLV and SLV were significantly reduced by ribavirin from 60% to 12% and from 18% to 3% respectively (Fig. 3).

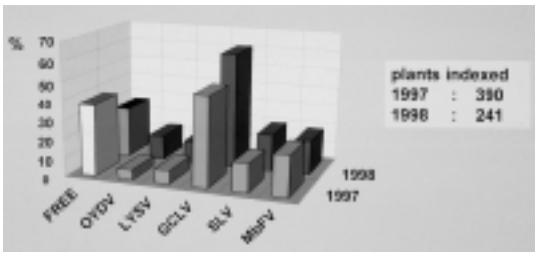

Fig. 2. Virus indexing on *in vitro* plants after meristem culture.

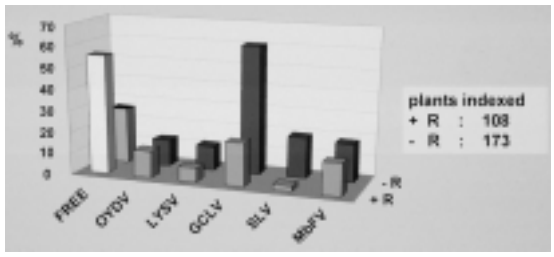

Fig. 3. Influence of ribavirin in the induction medium on virus elimination through meristem culture.

Furthermore, with ribavirin it was possible to obtain virus-free plantlets from larger meristem explants, whereas no virus elimination could otherwise be obtained. With 100 mg/L ribavirin, all regenerates revealed to be virus-free. They were, however, hyperhydrated and therefore not usable. The combination of chemotherapy and thermotherapy of bulbils resulted in an increase of virus elimination up to 84%. OYDV, LYSV and SLV could be totally eliminated and the number of GCLV and MbFV infections were reduced to 10% and 12% respectively.

At present, the virus-free *in vitro* collection of garlic comprises 98 accessions. The plants are free of OYDV, LYSV, GCLV, SLV and allexiviruses. The major part of these plants is maintained *in vitro.* Several accessions are grown also in the greenhouse or in the field under protected conditions. The complete results of this work are reported in the Final Technical Report of the Project GEN RES CT95-20 and in Senula *et al.* (2000).

Conclusion

In principle, it is possible to obtain virus-free *in vitro* plants from virus-infected vegetative plant material. The virus elimination efficiency varies depending on the viruses. Especially the economically important viruses OYDV and LYSV can be eradicated to 90% via meristem culture. Using additional chemotherapy and/or thermotherapy, the carlaviruses can also be eliminated up to 90%.

The *In vitro* Group of the IPK genebank was able to prove that *in vitro* cultures can be used to minimize the risk of pathogen distribution. Therefore, germplasm should be moved as virus-free *in vitro* cultures in all cases where this is possible. This strategy is however limited by some factors such as genotype dependence and the time span required to obtain the *in vitro* cultures, as well as their transfer back to soil conditions. Furthermore, the labour

input and insufficient technical equipment may be, in some genebanks and other institutions, obstacles to this type of management.

Taking into account all advantages and limitations of *in vitro* maintenance, it can therefore be suggested that *in vitro* maintenance should be favoured to preserve especially valuable collections, e.g. core collections established in various project programmes, in order to maintain an always protected virus-free nucleus of accessions. This nucleus should be maintained in high quality conservation conditions by several sufficiently equipped institutions, in order to provide high quality material for broader use. For other (non-nucleus) material, the need for rapid exchange may still, in the future, require movement of "traditional" vegetative plant parts, combined with minimum quarantine measures.

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The European *Allium* Database

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The first meeting of the ECP/GR Working Group on *Allium* was held at the Institute for Agrobotany (RCA), Tápiószele, Hungary in May 1984. This meeting agreed on a standard set of passport fields for use in a European *Allium* Catalogue. RCA and the Genetic Resources Unit, National Vegetable Research Station, Wellesbourne (now Horticulture Research International—HRIGRU) agreed to collect and format data into the standard format allowing the HRIGRU to develop the European Catalogue.

By the second meeting of the ECP/GR Working Group on *Allium* held in Olomouc, Czechoslovakia in 1986 a number of collection curators had provided passport data for inclusion in the ECP/GR European *Allium* Database (EADB). The meeting report provided a summary of the data registered in the EADB, which totalled 3187 accessions from 13 countries.

The report also detailed the results of an ECP/GR questionnaire survey sent to *Allium* specialists worldwide on the value of characterization descriptors for selecting germplasm from collections. The results were extremely interesting and have largely defined the minimum characterization descriptors utilized by the Working Group on *Allium* since.

Following a recommendation from the second meeting, HRIGRU published and distributed to *Allium* national programme coordinators a *Catalogue of European* Allium *Collections* in June 1988. The catalogue contained data of the significant collections of *Allium* in Europe with a total of 3853 accessions from 23 collections in 20 countries. The catalogue also contained a useful list of accepted taxonomic names prepared by P. Hanelt, IPK-Gatersleben. The availability of the Catalogue and the EADB data in computerized form was "advertised" when the Group held its third meeting in conjunction with the 4th Eucarpia *Allium* Symposium held at HRI, Wellesbourne in September 1988.

During the next 10 years the EADB was updated intermittently with new and edited national programme data. At the sixth meeting of the Working Group on *Allium* in Plovdiv, Bulgaria, October 1997, the Group reviewed the proposal for a standard set of multicrop passport descriptors defined by IPGRI IT experts and the central crop database managers in Budapest in 1996. The proposal to use the multicrop passport descriptors for the European central crop databases and for data exchange was supported unanimously. The Group chose to add 4 *Allium* specific descriptors to the database format as optional fields for national curators and collection managers. HRIGRU subsequently edited the existing national data sets to conform to the multicrop passport/*Allium* descriptor format by early 1998.

The development of the EADB during the period 1996-2000 benefited from the involvement of EU *Allium* genetic resources specialists in the EU GEN RES 20 *Allium* project. HRIGRU, supported by the UK Ministry of Agriculture, Fisheries and Food and the GEN RES project, developed the EADB as a downloadable file (Access or Excel) on the HRIGRU Web site linked to the ECP/GR Information Platform. The 1999 version of the EADB contained data on 8400 accessions from 20 institutes in 13 countries and the Nordic Gene Bank as per the attached list (Table 1).

In preparation for the current meeting on vegetatively propagated *Allium* material there was an obvious opportunity to update the European *Allium* database, in order to provide the necessary foundation for the discussions. Therefore a request was distributed to all members of the ECP/GR Working Group on *Allium*, previous data donors and other collection managers, asking for updates on the content of existing national/institute data or

to confirm that existing EADB content were still valid. An updated descriptor format was also distributed as a Word file (EADB-DESCRIPTORS2001.DOC) defining only one significant change to the EADB-1999 format, namely the removal of the REGION field. Data donors were requested to update their own data by incorporating any REGION data into the COLLSITE field.

Before the meeting 8 collection curators (7 national programmes and NGB) responded by sending data or reaffirming existing data (Table 1), so that before the meeting the new proto-database EADB-2001 contained 7374 accessions. The vegetative *Allium* subgroup agreed that other curators should be encouraged to update their national data in order that the EADB-2001 can be used to identify the "most original samples" and putative duplicates in collections.

Table 1. Data donors to the ECP/GR European *Allium* Database, 1999 and 2001

INSTCODE	Address of institute	1999	2001
BGRGORNA	Experimental Station for Vegetable Crops, Gorna Oryahovitsa, Bulgaria	+	
BGRIIPR	Institute of Introduction and Plant Genetic Resources, 4122 Sadovo, Bulgaria	+	
BGRPLOVDIV	Institute of Vegetable Crops 'Maritsa', 32 Brezovsko Shosse, 4003 Plovdiv, Bulgaria	+	
CHERAC	Station Fédérale de Recherches Agronomique de Changins, 1260, Nyon, Switzerland	+	
CZEOLOMOUC	Vegetable Section, Genebank Department, Research Institute of Crop Production, Slechtitelu 11, 78371 Olomouc-Holice, Czech Republic	+	
DEUBGRC	Federal Centre for Breeding Research on Cultivated Plants – Gene Bank, Bundesallee 50, 38116 Braunschweig, Germany	+	
DEUGAT	Institut für Pflanzengenetik und Kulturpflanzenforschung, Correnstrasse 3, 06466 Gatersleben, Germany	+	+
ESPDGIFA	Centro de Investigación y Desarrollo Agrario, CIFA Alameda del Obispo, Apartado 4240, 14080 Córdoba, Spain	+	+
ESPDGAZARA	Agricultural Research Service, Horticulture Department, Zaragoza, Spain	+	
ESPPOLVAL	Departamento de Biotecnologia, Universidad Politecnica de Valencia, Camino de Vera 14, 46022 Valencia, Spain	+	
GBRHRIGRU	Genetic Resources Unit, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, United Kingdom	+	+
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Research

Experience of in vitro *storage and cryopreservation of* Allium *at IPK, Gatersleben, Germany*

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Introduction

Genebanks dealing with vegetatively propagated material are all too familiar with the many problems and obstacles which render the maintenance of this material more difficult than that of seed-forming material. Labour, diseases and sample exchange are the principal sources of difficulty. Therefore, any measures helping to make preservation simpler, more cost-effective and safer are highly welcome. Laboratories can contribute to achieving this goal by adapting a spectrum of conservation methods. *In vitro* slow-growth storage and cryopreservation are important in this respect.

The first steps in this direction were made at the Gatersleben Genebank in 1990, which was a year of many changes. Work on *in vitro* culture of *Allium* was started with *in vitro* material derived from a haploid induction programme in onion. Haploid plants are seed-sterile and can be used as model cases. Thus, an onion clone has been permanently maintained as *in vitro* culture since 1987. Other seed-sterile material was received from a crossing programme which created distant *Allium* hybrids with the aid of embryo rescue culture in 1991-1993. In 1992, the introduction of 50 garlics, 10 shallots, *A. hookeri* and *A. chinense* into *in vitro* culture using shoot tips explants marked the start of a small project financed by the state of Saxony-Anhalt. Further support was provided by the EU-funded GEN RES Project CT95-20 from 1996 to 2000, which funded the employment of a scientist for meristem culture and ELISA tests, leading to the establishment of a virus-free *in vitro* collection. Another EU project (FAIR CT95-465) resulted in the possibility of storing *in vitro*-produced bulblets of *Allium cepa* at -1°C for longer periods (at least 1 year) without manipulation. The latest input came from a special IPGRI-funded project in which IPK staff, together with Zanetta Makowska, from RIVC Skierniewice, Poland, received a 2-week training in cryopreservation techniques at the Fruit Tree Institute at Ciampino-Rome, Italy, from Dr C. Damiano and his staff.

It is important to mention that most of the input has come from temporary projects, and the continuation of these activities is always endangered by the lack of permanent staff able to manage these growing resources. Therefore, many of these potentially very beneficial techniques cannot yet be utilized to their full potential, which will become more and more of a priority in the future.

In vitro culture of *Allium*

The most obvious advantage of *in vitro* storage is the separation of these cultures from pathogens in the field, accompanied by options to clean the material from viruses already accumulated in the plants (Havránek 1972; Senula *et al.* 2000). Another is the reduction of space requirements for the maintenance of the material, which is indeed an advantage, depending on the storage capacity of the respective genebank. There is only a limited advantage with regard to labour, because care has to be taken of the plant material which is metabolizing, growing and developing even in slow-growth culture; culture medium has to be continually prepared and microbes can be harmful when entering the tubes or breaking out from latent infections.

Nevertheless, the sum of these factors leads to a progress in maintenance of the samples. Taking into account that members of the genus *Allium* possess a broad range of diversity with respect to physiological and morphological characters, due to a broad range of ecological conditions in their local sites, from continental desert ephemeroids to oceanic wet

meadow perennials, the development of *in vitro* storage protocols has to take into account many different factors. Garlic and shallot are the most studied groups, in which cycles of warm and cold cultivation could be used in the IPK laboratory conditions.

Explants can be taken from various organs:

1. Basal plates have the advantage of being present throughout the year. However, it should be remembered that their physiological status varies with the season. The best growth is obtained in early spring when the material emerges from the winter rest period. Some species can have a strong flower formation tendency *in vitro* after having been vernalized, which reduces the chance of establishing permanent micropropagation. Shallots and garlic have more than one shoot tip per basal plate, depending on the genotype. The material is very often endangered by severe infections due to the location of the bulb in the soil. Warm water treatment and very thorough washing and disinfection may be necessary in such cases.
2. Young growing inflorescence stalks, mostly located still in the bulb. This is a limited source with respect to duration of availability, number of explants and morphogenesis, which goes prevalently through adventitious bud formation. The risk of infections is the same as in point 1 above.
3. Particular success has been described in the literature (Haque 1997, 1998) using garlic root tips. However, the morphogenesis here goes more or less through a callus-like phase which endangers the genetic stability.
4. The basal part of the young inflorescences gives a very good source material for explants. However, access is limited to few weeks before the opening of the spathe.
5. Bulbils are one of the best sources in all accessions that form these propagules in their inflorescences. Sometimes there is a period of dormancy after harvest that can be broken by cold pre-treatment. In Central Europe, bulbils are best used between December and May of the following year. The advantage is that they are much cleaner than soil-borne organs and that they are often available in large homogeneous quantities. Possible higher genetic instability of bulbils has been discussed, but this has not yet resulted in any real evidence of higher genetic deviation.
6. In accessions forming flowers, the reactive tissue around the nectary glands can give regenerants in some limited cases.

Cases 1 and 5 above can be used to establish meristem cultures for virus elimination, whereas the other cases can be recommended as additional options for rare and endangered material, where every possible mean of rescuing the material should be considered before touching the proper meristems, which could result in complete loss of the samples if the operation is unsuccessful. In these cases adventitious shoots are formed in the respective tissues directly or via a transient callus phase, so that finally micropropagation is possible from these newly formed shoots.

Allium clones maintained *in vitro* at IPK are listed in Table 1.

Table 1. *In vitro* collection of *Allium* material in the Gatersleben Genebank as of May 2001

Material	No. of accessions
Haploid/diploid regenerants of an onion haploid programme together with their donor genotypes	21
Distant *Allium* hybrids, mainly of onion and wild relatives	82
Garlic (untested)	27
Garlic (virus-free)	98
Great-headed garlic	1
Shallots	21
A. chinense	3
A. hookeri	2
Diverse wild species	49
Safety-duplicates (mainly garlic)	68
Total	**372**

The Gatersleben group experimented with several conditions: *in vitro* cutting and splitting; hormones after the cutting phase and in the permanent culture; extended warm cultivation; high sucrose content in the medium; various temperatures; storage of small meristematic clumps, bunches and *in vitro* formed bulblets. After a series of steps to learn more about the *in vitro* behaviour of *Allium* in our laboratory and elsewhere (Bhojwani 1980; El-Gizawy and Ford-Lloyd 1987; Moriconi *et al.* 1990; Kahane *et al.* 1992; Keller 1993; Viterbo *et al.* 1994; Mohamed-Yasseen *et al.* 1994, 1995; Keller *et al.* 1997; Nagakubo *et al.* 1997; Keller and Senula 2000; Kästner *et al.* 2001), the following recommendation could be made:

The culture should, if possible, be initiated by way of meristem culture, including a check to confirm that it is virus-free. If this is not possible, and the material is rare or endangered, all other options can be used to rescue the material with subsequent meristem cultures, eventually supported by thermotherapy and/or virazole at a later stage.

A schematic description of the *in vitro* culture cycle is given in Fig. 1. The culture medium can be BDS (modified B5, Dunstan and Short 1977) or MS (Murashige and Skoog 1962). The permanent culture should be conducted on hormone-free medium to avoid hyperhydricity and reduce the risk of somaclonal variation. Each explant will pass a cutting phase after which a subculture could be inserted on a medium with hormones, e.g. 0.1 mg/L NAA and 0.5 mg/L 2iP (Bhojwani 1980) or 0.1 mg/L IAA and 0.1 mg/L kinetin (Moriconi *et al.* 1990). The temperature in the warm phase can range from 20°C to 25°C without substantial differences. The cold phase can be from 2°C to 4°C. Culture in smaller tubes (reagent tubes of any size containing one or two explants) is better than the use of larger jars because of the infection risk. The optimal subculture duration is 2 months in the warm phase and 12 months in the cold phase. Due to organizational reasons, extension of the warm subculture phase was often necessary: it can be extended up to about 8 months and is limited by the drying of the agar. Previous results showed that prolongation of the warm phase can deepen the dormancy of *in vitro* bulblets in the cold phase (Keller *et al.* 1997), an often desired phenomenon. The extension of the cold phase may be critical because of the increasing hyperhydricity. A thorough check of the cultures is then necessary because the first stages of hyperhydricity may be reversible. Later on, the irreversible degradation due to hyperhydricity may cause the loss of the cultures.

Regardless of the method used, a splitting of each *in vitro* clone in two or three parts is necessary for safety reasons. Each of the respective clonal subsets should be treated as independently as possible with respect to the time of each treatment, media charge, cultivation site, etc. Thus the clonal subsets may function as safety-duplicates for each other.

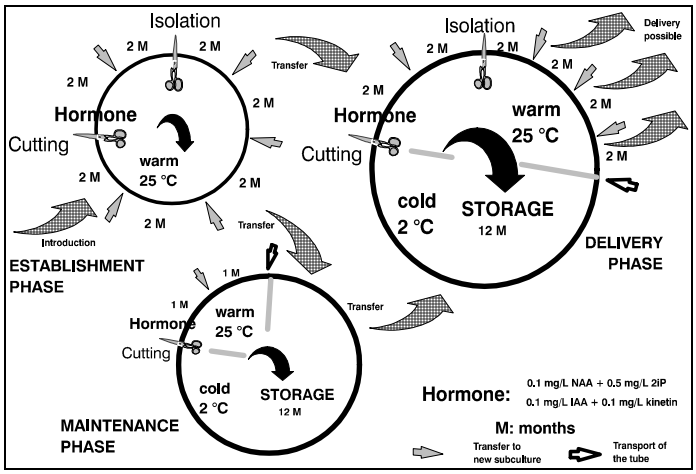

Fig. 1. Culture cycle for the *in vitro* maintenance of *Allium.*

Cryopreservation of *Allium*

Storage of meristem explants in or above liquid nitrogen has been shown to be potentially the safest and most cost-effective method of long-term maintenance of a large collection. During the storage phase no contamination and no metabolic processes can take place, thus reducing the need to manipulate the material, as long as the basic requirements (temperature constancy) are permanently met. The usual risks and specific requirements of *in vitro* culture however apply to the pre- and post-storage phases.

Cryopreservation exposes the explants to various stress situations resulting in difficulties that have to be overcome by cryoprotective treatments and recovery culture conditions. This is why cryopreservation is still in the developmental stage and progress reports are still limited to some laboratories.

For assessing the cryopreservation process, non-frozen controls must always be conducted in parallel with the experiments. Two parameters can be distinguished: (i) survival, i.e. persistence of the green and vigorous appearance after rewarming; sometimes there is some degree of swelling and leaflet extension, but no growth of new shoots can be observed; (ii) regrowth, i.e. development of new shoots some days or weeks after rewarming (Fig. 2).

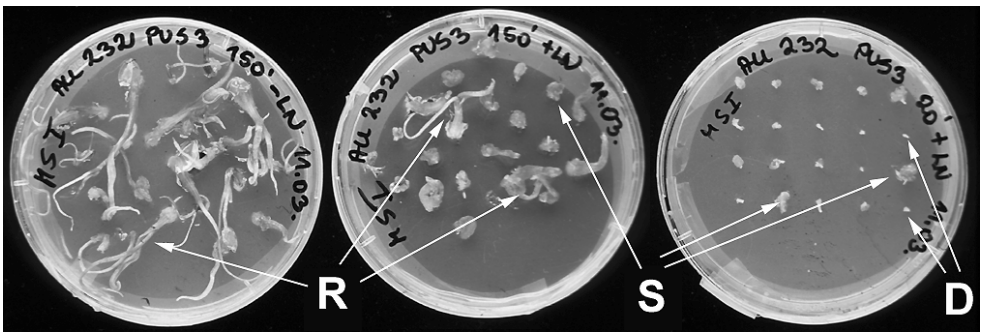

Fig. 2. Definition of the different growth characters 3 weeks after cryopreservation. (D = dead explants; R = regrowth; S = survival)

The following source organs have been used so far for cryopreservation in garlic:

1. Cloves: they provide the more vigorous and more easily freezable shoot tips so far tested. The disadvantage is that the higher the degree of infection, the lower the number of usable explants compared to the use of other plant parts. At Gatersleben, the high responses in survival and regrowth reported in the literature (Hannan and Garoutte 1996, 1998; Niwata 1995) was confirmed (Makowska *et al.* 1999).
2. Bulbils: these are very often accessible in large quantities. In our experiments, however, a high degree of genotype dependence has so far resulted in restriction of their usability to accessions with large bulbils.
3. *In vitro* cultures: their advantage is the possibility to use directly virus-free material. So far problems still exist at Gatersleben in obtaining sufficiently high percentages of survival and regrowth from *in vitro*-grown material. IPK is presently experimenting with methods to dehydrate the *in vitro* explants to make them freeze better (Keller 2001).

The cryo-collection

It would be relatively easy to start a cryo-collection from clove explants. However, such a collection would be of limited use because clove explants are not necessarily virus-free. Even a virus check of clove material obtained from partners with a virus-free declaration showed that in some cases the material was in fact infected. Therefore, IPK decided to

continue working on the improvement of cryopreservation methods for *in vitro* material and, in the meantime, store only a small test collection of some accessions (Table 2) frozen with the same safety standards as the existing IPK potato cryo-collection (Schäfer-Menuhr *et al.* 1994, 1998), i.e storage of three independent sets of 120 explants each.

Table 2. The preliminary cryo-collection of garlic in the IPK genebank

Accession number	3 sets stored	2 sets stored	1 set stored
All 290	x		
All 508	x		
All 525	x		
All 651			x
All 841	x		
All 937		x	
All 1292			x

The plants' development is recorded several times in a standard clone by counting survival and regrowth, as well as scanning the Petri dishes 2, 5 and 8 days after warming; by recording the appearance of the plantlets after removing them from the culture tubes after 4 months; by scanning or photographing them; by counting the potted plants after 6 months, and then in the field after 18 months; and finally by assessing the bulbs after harvest (22 months after the experiment). Potted plantlets are similar to those from bulbils. Two-year-old plants have the same vigour as bulbil-derived plants and are ready for morphological evaluation. They would also be ready for direct use.

Concluding remarks

Both *in vitro* culture and cryopreservation are making good progress at Gatersleben. Thus, they can be offered as alternative methods for germplasm preservation. However, the following factors should still be considered:

1. Genotype dependence: the genebank curators, especially those who work with *in vitro* cultures, know that some genotypes do not respond as well as others. Genebank work is always the subject of compromises in order to keep the largest possible number of accessions while maintaining a low level of variability with regard to maintenance methods. Therefore, it should be borne in mind that there will always be a remainder of recalcitrant accessions for which it is not worth increasing any efforts.
2. Latent infection: being bulbous plants, of which the meristematic parts are situated close to the soil surface, the degree of infection may be rather high and, in some genotypes, latent infection may persist for long periods (Fellner and Havránek 1994; Fellner *et al.* 1996). They can break out when the cultures are placed under stressful conditions, such as cryopreservation treatments. Thus, *in vitro* cultures should always be safety-duplicated within IPK. A higher number of small tubes containing one or two explants should be favoured instead of a smaller number of large culture jars containing more explants. Latent infections are also a possible cause of losses during cryopreservation; therefore the number of explants to be frozen should not be too low.
3. Time requirements: recovery from cryopreservation requires some time before the plants can be used like normal field-grown material. Cryopreservation is, therefore, not a suitable method for active collections designed to have a quick turnover, although the safety-supporting role of cryopreservation should always be emphasized. This may not be sufficient to ensure the adoption of this methodology in times of limited resources. In some cases, even *in vitro* storage may cause delay in the usability of samples because regenerants derived from *in vitro* culture may behave like seedlings, which grow relatively slowly in some *Allium* species.

Nevertheless, regarding the increasing threat to the conservation of genetic resources, the safety aspect should evidently be placed at the top of any priority list. Therefore, genebanks working on *in vitro* storage and cryopreservation are on the right track to contributing to the development of these conservation methods.

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Cryopreservation at the Research Institute of Crop Production, Czech Republic

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The field or orchard plant depositories demand large inputs of labour and reclaimed land. In the field collections, there are high risks of losing accessions due to unfavourable environmental conditions, diseases and other hazards. An alternative to field collection of vegetatively propagated plants is the conservation of plant tissues or whole plants in *in vitro* conditions in normal or limited growth. *In vitro* plant conservation is not useful for long-term storage because of the need for frequent subcultures, contamination, higher probability of mutation, labour costs and risk of human errors associated with tissue culture manipulation.

This presentation concerns methods for cryopreservation of plant shoot tips or meristems, maintenance of the samples at ultra-low temperatures and their successful recovery to growing plants.

Plant tissue cultures

Plant tissue cultures provide a useful tool for growing and reproducing whole plants, or plant parts, in artificial media. They are essential for preparing plants for cryopreservation. Tissue culture offers many important advantages, such as the possibility to isolate and maintain plant clones. During *in vitro* cultivation, the tissue cultures are easily cleaned of any contamination by fungi and bacteria strains by discarding the contaminated plants from *in vitro* cultures.

Plant tissue cultures can also be used for mass propagation. However, certain difficulties with the use of tissue culture should be mentioned:

1. Some plant tissue cultures, at a certain stage of development, may be subject to hyperhydricity (Luthar and Bohanec 1999) which results in malformation, and affected tissues typically degenerate and die.
2. Acclimatization is another common problem in tissue culture. Once entire plantlets are successfully regenerated, they must be acclimatized before being transplanted. The plants adapted to high humidity (close to 100% relative humidity) can be killed during their transplanting to low humidity into a growth chamber or greenhouse. This problem can be avoided by a gradual reduction of relative humidity or by giving them time to adapt to low relative humidity.
3. The third problem in tissue culture is the chemical composition of the culture media, which can greatly affect the growth of plantlets. Each cultivar can have different nutritional requirements during its development and genotype-specific changes in the basal medium may be beneficial.

Tissue cultures are an important biotechnological step before cryopreservation. They provide a source of disease-free stock material suitable to undergo acclimatization steps before freezing. After freezing, if some cells are injured by liquid nitrogen, tissue cultures are commonly used for recovery of plants from freezing temperatures and for their regeneration.

Cryopreservation

Cryopreservation is based on the reduction and subsequent interruption of metabolic functions of biological material stored at ultra-low temperatures. The aim of cryopreservation is the cryogenic preservation of plant parts for extended periods without genetic changes occurring, and subsequent recovery of normal plants with unaltered

characteristics and biosynthetic ability, which has important implications in plant breeding programmes and/or genetic engineering. At the temperature of liquid nitrogen (-196°C) almost all metabolic activities are arrested and plants can be maintained in this suspended but viable state for extended periods.

Cryopreservation methods

While routine cryopreservation of microorganisms, zygotes and animals derived from zygotes is possible, the cryopreservation of plant cells is far from routine and different protocols for individual species of plants are often necessary. Most plants, including those that are vegetatively propagated, cannot survive freezing and thawing procedures from cryogenic temperatures without cryoprotective agents and special pre-treatment procedures. Many cryopreservation methods are used, though recently, the most widespread methods in use are based on vitrification. Vitrification is the conversion of plant tissue into an amorphous or glassy state of matter. Glass is considered a liquid with the viscosity of a solid (Hirsh 1987), or a viscous liquid or a non-crystalline solid (Williams and Leopold 1995). Low diffusion in a glassy state is an important property especially for long-term storage of biological material.

There are two main methods to induce vitrification in plants:

-The first method uses the cryoprotectant to induce a glassy state during freezing to ultra-low temperatures.

-In the second method, the content of plant cells is concentrated by dehydration to the final concentration corresponding to the glassy state.

Vitrification by plant vitrification solution

A number of cryoprotective protocols were developed for direct protection of plants by cryopreservation, where the deleterious effect of dehydration is mitigated by the presence of cryoprotective agents. Plant parts are brought to equilibrium with solutions containing cryoprotective agents in different ratios. The most used cryoprotective agents are dimethylsulfoxide (DMSO), ethylene glycol, glycerol, sucrose and sorbitol in various combinations and concentrations. Widely used concentrations of cryoprotective substances are in the range of, for example, the plant vitrification solution 2 (PVS2) (Sakai *et al.* 1990), which contains 30% glycerol, 15% DMSO, 15% ethylene glycol in 0.4 M sucrose. Apices of *Allium* treated with PVS3 solution had a much higher regrowth rate than those treated with PVS2 solution (Makowska *et al.* 1999). The vitrification procedure with a vitrifying agent at ambient or reduced temperature for a certain period of time is applied in cryopreservation of shoot tips. The length of the treatment with the vitrification solution is very important, because the optimum time for glassy state induction is very close to the time when irreversible injury is caused by the vitrification solution. If the concentration of the vitrification solution is highly toxic for a particular plant or a cultivar, the survival of treated plants is low; therefore vitrification solution treatment at low temperatures can be used. In some cases the survival of plants in PVS2 at low temperatures can be 6 to 10 times longer. For high concentrations of a vitrifying solution, plant parts are quickly frozen up to the temperature of liquid nitrogen.

Vitrification by encapsulation-dehydration method

Another type of vitrification is obtained with the combination of a dehydration pre-treatment and/or with encapsulation in alginate gels. These cryoprotocols are more sparing to plants because they avoid the pre-treatment with highly concentrated vitrification solution. The glassy state in plants can be reached by simple dehydration (Bilavcik and Zamecnik 2001; Grospietsch and Zamecnik 2001; Zamecnik *et al.* 2001). During dehydration the vitrification agents reach the concentration of vitrification. Survival and regeneration rates obtained with two vitrification methods are shown in Table 1.

Table 1. *Allium* shoot tips cryopreserved by two vitrification methods (3 independent experiments with 200 shoot tips in each)

Vitrification method	Survival (%)	Regeneration (%)
Plant Vitrification Solution (PVS2)	89	82
Encapsulation-dehydration	48	45

Plant recovery from an ultra-low temperature is a further important step of cryopreservation. Thawing of cryopreserved plants should be rapid to avoid crystallization of ice, which leads to lethal damage of plant cells.

The disadvantage of using the PVS solution is the need to subsequently remove it from the plants. The vitrification medium could be highly toxic for plant cells at room temperature. Plants are therefore incubated into media suitable to remove the vitrifying solution. After removing the vitrification solution from the surroundings of the cells, shoot tips are washed out with a gradually decreasing concentration of sucrose solutions. When the osmotic potential of the surrounding solution is in equilibrium with plant cell water potential, the plants can be transferred to the favourable conditions for their recovery.

Establishment of a cryobank in the Czech Republic

In order to transfer *Allium* plants to *in vitro* conditions, shoot tips obtained either from cloves or bulbils will be used. After testing for viruses, virus-free plants will be multiplied in *in vitro* conditions. After multiplication of the plants, shoot tips will be cryopreserved following one of the above-mentioned cryoprotocols. Tests on plant viability and regeneration will be made in the first week following cryopreservation.

Elaboration of a cryoprotocol

It will be a part of EVIGEZ, the Czech plant genetic resources documentation system. EVIGEZ contains passport, characterization, evaluation and storage data of *Allium* accessions. An important item of the cryoprotocol is the information about the culture media (content and concentration of phytohormones for successful regeneration). The comparison of the percentages of regeneration after 1 week and after 1 year will be essential for deciding the timing of a second or third freezing. The safe range of storage temperatures will be defined on the basis of measurements of glass transition temperatures. When the temperature rises above the safe storage temperature, problems with the regeneration of the plants can be expected. Cryobiologists must prepare a cryoprotocol suggesting the most suitable thawing method and regeneration protocol, according to the latest level of knowledge. Although several improvements of the technology can be expected during the storage time of the plants, it might not be appropriate to introduce a new thawing method if an old freezing method was applied.

The sequence of manipulations involved is summarized in Fig. 1.

Strategy to overcome problems and constraints

The *Allium* clones with low survival rates in liquid nitrogen will be stored in *in vitro* conditions until improved cryopreservation methods for these genotypes have been satisfactorily developed. Antibiotics will be used only in rare cases when intrinsic contaminations appear. The Alarm Monitoring System monitors the temperature inside the Dewar flask. RICP has a contract with the LINEQ Company, which is able to supply the cryobank with liquid nitrogen within a few hours. The cryobank is under permanent control by a security agency.

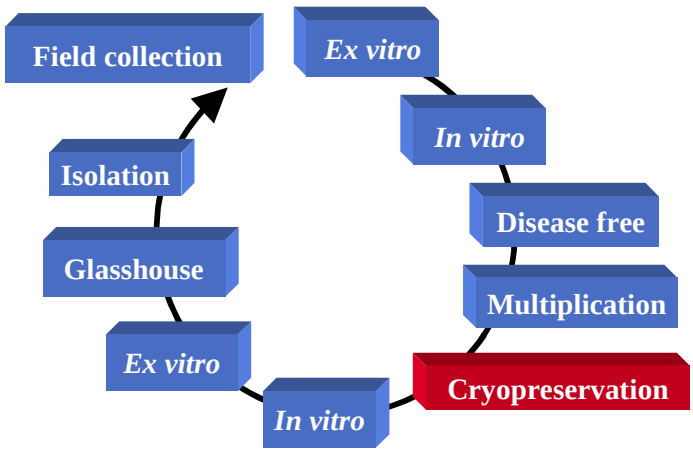

Fig. 1. Manipulation and storage of *Allium* genetic resources.

Cryobank storage technology

Allium shoot tips will be stored in cryovials in different Dewar flasks to avoid the risk of losing vacuum. All cryovials, cans and Dewar flasks will be identified by a bar code. Different scanning calorimeter will be used for exact measurement of glass transition temperature. The glass transition temperature will be used for development and improvement of methods for genotypes with a low percentage of survival, control of the rate of freezing for originating glasses and avoiding crystallization, measurement of glass transition changes in time, and characterization of glass transition temperature and heat capacity of plant meristems.

Expected benefits

- **Scientific**

Up to now, most cryopreservation procedures and techniques have been based on empirical experience. Application of thermodynamics to cryopreservation is expected to improve the conservation of germplasm. The goal is to run the cryoprocesses under well-defined rules based on the latest scientific achievements in the field of low-temperature thermodynamics.

- **Economic**

Formerly neglected *Allium* varieties will be characterized and information on potentially useful varieties will be made available to the users. The Czech *Allium* breeding company SEMPRA, by utilizing the results of this project, will produce superior varieties and strengthen its position in its sector. Special traits and chemical compounds of germplasm of *Allium* genotypes stored today are expected to become useful in medicine in the future.

- **Environmental**

When *Allium* breeders and scientists will use accessions from the cryobank, new *Allium* varieties will be released with improved resistance against pests and diseases and/or improved nutritional composition. Appropriate varieties targeted for organic farming should be identified.

Overview of *in vitro* and cryopreservation research for long-term conservation of vegetatively propagated *Allium* collections in the Czech Republic

Experience gained at RICP, Prague in the application of micropropagation methods for different cultivars and species

- Identification and optimization of media, growth regulators and other growing factors for micropropagation of a broad range of genotypes within all studied species (*Solanum* sp., *Allium* sp., *Humulus lupulus, Malus* sp. and *Pyrus* sp.)
- Determination of appropriate conditions for establishment, multiplication and rooting among cultivars within the five studied species
- Solving problems with internal and external bacterial, yeast and fungal contamination.

Development of low-temperature or slow-growth storage techniques for *in vitro* cultures (i.e. 4°C or 22°C)

- Specific conditions for slow-growth storage techniques in *Allium* sp., *Malus* sp. and *Pyrus* sp.
- Testing conditions during slow growth and choice of the optimal time for subsequent cryopreservation
- Cold-stored collections are monitored to determine the storage life and optimal conditions for individual accessions' regrowth and regeneration *in vitro*.

Development of cryopreservation methods for successful storage of germplasm (shoot tips, meristems and pollen) in liquid nitrogen

- Evaluation of pre-treatment techniques, application of cryoprotectants, freezing protocols and optimization of recovery stages
- Screening of convenient genetic resources (germplasm) to determine the applicability of a specific cryoprotocol to a broad range of genotypes
- Development or adaptation of techniques for the range of genotype response within a species
- Long-term storage in liquid nitrogen is now established for the *Solanum* collection.

Application of biophysical methods in plant cryopreservation for monitoring thermodynamic changes at the molecular level under low and ultra-low temperatures

- Application of differential scanning calorimetry for detection of biological glass using determination of changes in heat capacity and glass transition temperature
- Differential thermal analysis for determination of phase transitions in plants
- Measurement of ice nucleation activity and ice propagation rate in plants
- Determination of LT50 by freezing in controlled freezers from 0°C down to –196°C.

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Statistical analysis of some quantitative characters of garlic accessions

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Introduction

In Bulgaria garlic forms and cultivars belong mostly to subsp. *vulgare*. Garlic productivity is a result of the direct action of each of the yield components and of their interaction. This interaction can be determined by the analysis of correlation between the characters of the bulb. This would be a useful theoretical basis for selection of starting material and could offer a correct guidance in the breeding process, that would take in consideration quantitative characters of garlic, which are genetically determined, as well as their variation with external factors (Bachvarov *et al*. 1990; Manuelyan *et al*. 1991).

According to Kampe (1967), quantitative characters are controlled by a greater number of genetic factors than qualitative traits. The bulb size is a quantitative character and largely depends on the photoperiodical reaction of the cultivar (Bachvarov *et al*. 1990).

The main purpose of this investigation was to establish the correlation coefficient between the bulb mass and the most important quantitative characters related to it, to be used as a theoretical basis for breeding (Neykov *et al*. 1992).

Material and methods

In this study promising garlic forms (subsp. *vulgare*) collected in the country were selected and were studied at IHC, Plovdiv (Table 1). For determination of the quantitative characters, 40 plants per accession were analyzed. Mathematical analysis of the data for 11 indexes (average) was performed by computer programs for data analysis (Barov and Iovcheva 1968; Draper and Smith 1973; Grandon 1979). Correlation coefficients were used to find out the regression equation describing the relation between the bulb mass and the parameters directly affecting it. The step regression method (Draper and Smith 1973) was used. This method consists in finding out an optimum multiple equation, since in each stage of the procedure two hypotheses are checked for inclusion or exclusion of particular variables.

Results

The evaluation data of 11 morphological characters are presented in Table 1. The bulb mass varies from 27.4 to 42.9 g. Accessions Nos. 115, 118 and 119 have heavier bulb mass, clove number and clove mass (Table 1). The correlations between some quantitative characters were established (Table 2). A significant positive correlation was found between bulb mass and bulb height, clove mass and clove number (r = 0.71; 0.51; 0.69). Plant height is positively correlated with pseudostem height and leaf number (r = 0.50; 0.90). Leaf number is positively correlated with pseudostem height, clove mass and clove number (r = 0.52; 0.74; 0.63) and negatively correlated with bulb skin number (r = -0.46). Bulb diameter is positively correlated with bulb length (r = 0.73) and negatively with pseudostem thickness (r = -0.45). Clove number and clove mass show positive correlation with the majority of the other parameters (r = 0.51-0.88) (Table 2). Since in many cases the positive effect of one character is hidden to a great extent or completely by the negative effect of other characters, the step regression method was chosen in order to find the best regression equation to describe the effect of various parameters on the bulb mass. As a final result a regression equation was obtained: $x_8 = 42.039 - 4.12 \cdot x_4 + 1.480 \cdot x_5 - 1.345 \cdot x_6 + 1.081 \cdot x_{11}$. This shows that the bulb mass (x_8) is dependent on bulb length, clove mass, leaf number and bulb diameter.

Conclusion

The bulb mass was found to show a significant positive correlation with bulb height, clove mass and clove number (r = 0.71; 0.51; 0.69). The results of this analysis will be helpful in selecting appropriate garlic accessions in future collecting and breeding programmes.

Table 1. Characters analyzed in the study of some garlic accessions from Bulgaria

		Character										
No.	**Origin**	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**	**X_6**	**X_7**	**X_8**	**X_9**	**X_{10}**	**X_{11}**
112	Razgradsko	50.90	15.32	11.17	6.67	32.36	40.72	0.79	27.39	3.11	8.87	21.57
113	Simeonovgra	51.65	16.50	10.27	6.35	32.80	43.40	0.75	29.50	2.82	9.57	23.95
114	Pobeda - Jambol	53.70	16.82	11.25	6.45	34.02	43.72	0.78	30.60	2.85	8.95	23.32
115	Suhodol-Burgas	50.10	12.27	11.50	6.40	36.65	48.42	0.76	42.62	3.25	10.67	35.27
116	Chernovo-Burgas	58.70	17.40	12.55	6.97	33.17	37.85	0.78	29.92	3.72	8.37	23.10
117	Sarnevo-Burgas	56.97	16.42	12.90	6.70	35.86	46.46	0.77	36.38	3.34	9.80	29.11
118	Livora - Burgas	56.05	16.67	11.70	6.60	35.89	48.84	0.74	42.92	3.94	11.27	34.90
119	Sarnevo - Burgas	56.10	16.95	12.10	6.80	36.52	43.27	0.75	42.41	3.82	11.13	35.03
120	Pravdino - Jambol	55.95	16.80	12.27	7.05	32.71	39.78	0.76	38.27	3.16	9.41	24.66
121	Malina - Burgas	56.62	15.97	12.92	6.80	32.13	43.69	0.73	31.37	2.73	9.68	24.10

Legend:

X_1 = Plant height (cm)
X_2 = Pseudostem height (cm)
X_3 = Pseudostem thickness (mm)
X_4 = Leaf number
X_5 = Bulb height (mm)
X_6 = Bulb diameter (mm)
X_7 = Index (H/d)
X_8 = Bulb mass (g)
X_9 = Bulb skin number
X_{10} = Clove number
X_{11} = Clove mass (g)

Table 2. Matrix of correlation coefficients between variables in garlic accessions from Bulgaria

	X_1	**X_2**	**X_3**	**X_4**	**X_5**	**X_6**	**X_7**	**X_8**	**X_9**	**X_{10}**	**X_{11}**
X_1	1.000										
X_2	0.507*	1.000									
X_3	0.420	0.138	1.000								
X_4	0.906**	0.520**	0.033	1.000							
X_5	-0.058	0.176	0.412	0.268	1.000						
X_6	-0.318	0.130	-0.449*	0.392	0.732**	1.000					
X_7	0.345	0.339	-0.183	-0.217	0.008	-0.068	1.000				
X_8	0.207	0.232	-0.254	0.188	0.710**	0.258	-0.002	1.000			
X_9	0.262	-0.222	-0.046	-0.464*	0.218	-0.126	0.176	0.409	1.000		
X_{10}	-0.183	0.506*	-0.160	0.743**	0.525**	0.612**	-0.188	0.514*	-0.279	1.000	
X_{11}	-0.145	0.405	-0.376	0.639**	0.799**	0.765**	0.034	0.696**	-0.020	0.882**	1.000

Legend: see Table 1

$x_8 = 42.039 - 4.12 \cdot x_4 + 1.480 \cdot x_5 - 1.345 \cdot x_6 + 1.081 \cdot x_{11}$

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Appendices

Appendix I. Background document on vegetatively propagated *Allium* genetic resources in Europe[17]

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Introduction

The Genus *Allium* contains a number of important vegetables belonging to a number of species. These crops are utilized in many ways and are of different economic importance. Some taxa have been used as vegetables and, in some cases, as medicinal plants for centuries. Several taxa are becoming more and more important as medicinal plants. The diverse range of utilization is mainly due to the very complex and heterogeneous structure of the genus *Allium* with respect to its biosystematics (taxonomy), the growth behaviour of taxa and horticultural parameters. The secondary genepools of the "major" species are becoming more important at present because of progress in breeding and biotechnology. On this basis more wild species have to be included into the measures taken to conserve the genetic resources of *Allium*.

A considerable number of taxa in the *Allium* genepool are represented by plants which have to be propagated vegetatively for several reasons:

1. Some groups are predominantly or almost exclusively seed-sterile, namely garlic (*Allium sativum*), great headed garlic and some other subgroups of the *Allium ampeloprasum* complex (leek relatives) and rakkyo (*Allium chinense*). Some of these taxa are of hybrid origin (top onions, the French grey shallot and others).
2. Some groups are traditionally propagated vegetatively and have partly lost their fertility. This is mainly true for shallot.
3. Some species have normal seed set in their original habitat. They are included in the European collections because of their relatedness to the important crop species. Their fertility is poor because of maintenance in inappropriate environmental conditions. Their *ex situ* preservation is, nevertheless, regarded as being important for Europe for various reasons (use in current and future breeding programmes, the original habitat is endangered, absence of access to the sites of origin). This is an especially heterogeneous complex of mainly wild species of the subgenera *Allium* (leek / garlic relatives) and *Rhizirideum* (relatives of onion, shallot and chives).
4. Finally, there is a group of species of mainly ornamental use, representatives of which are integrated into some of the larger collections because of taxonomic interest. There are numerous species which do not have secured seed set because of adverse environmental conditions. They are, therefore, also maintained mainly vegetatively.

This heterogeneity of the genus has been emphasized above because it is the major reason for many problems associated with the conservation of genetic resources in the genus *Allium*. The seed-fertile accessions can be stored in seed genebanks relatively cheaply. Seed propagated taxa are, therefore, better represented than vegetatively propagated material in the European genebanks and thus available to users. The maintenance of accessions vegetatively is extremely expensive, time consuming, requires continuous input and has associated phytosanitary risks, and in many cases is subject to quarantine requirements for the movement of both field and *in vitro* material.

[17] (document prepared March 2001)

The problem

The historical overview below shows that considerable effort has been put into the conservation of vegetatively propagated *Allium* material by ECP/GR since the mid-1980s. Many of these actions were agreed as inputs in kind to ECP/GR by the respective national programmes. Thus there are no formalized agreements in place for the maintenance or safety-duplication of vegetatively propagated *Allium* taxa. There is an urgent need to discuss the technical inputs and political/financial requirements to ensure adequate maintenance and backup of the vegetative collections.

In addition to the collections mentioned above, there are other collections of vegetative material existing within national programmes that are considered to be important scientifically. In the present situation, it is doubtful whether the concept of maintaining "long-day" and "short-day" safety-duplicate collections will ever be possible for all such material. So new solutions have to be found for this particular problem. It is possible to envisage an inter-institutional network of vegetative *Allium* collections that agree on mutual exchange of information and, step-by-step, the exchange of material under bilateral duplication agreements. Such an arrangement would require significant inputs in terms of staff and resources compared to the "black box" arrangements for seed collections.

The EU GEN RES *Allium* project contributed significantly to the collection and maintenance of vegetatively propagated material in Germany and Spain for garlic, and in Greece for wild taxa. However, this project ended on 31 March 2000. The current situation regarding a follow-up programme in the GEN RES format, i.e. funds dedicated to PGR core functions not in competition with research, is not clear. It is difficult, therefore, to see who will fund the future maintenance of vegetative collections developed with an 'international' label such as those under the auspices of the ECP/GR Programme and/or the EU GEN RES project.

It was agreed at the ECP/GR Vegetables Network Coordinating Group meeting (Vila Real, Portugal, May 2000) that it is essential that collection curators meet to discuss these problems. The objective of these background notes is to provide the curators and National Coordinators with a clear overview of the problem. Where possible, we would like curators to come to the meeting having discussed the situation with their National Coordinator and to be in a condition to clarify the current position in relation to management and financial support of the vegetative *Allium* collections within their national programme. It will be even more helpful if they can provide an indication of future intentions for existing collections.

Objectives of the proposed Small Technical Meeting

- To update the survey on vegetatively propagated *Allium* accessions per country, including:
  - passport data
  - a definition of current financial arrangements for maintenance of the collections
  - *Allium* group (garlic, shallot, leek relative, top onion, wild species)
  - specify maintenance regime (genetic resources conservation proper, perennial maintenance for permanent seed collection, reference collection for research, reference collection for variety protection, breeding collection, others)
  - an assessment of disease status
- To identify "hot spots" of risk in the various collections
- To define measures for safety-duplication and coordination:
  - minimum requirements;
  - offers of action (continued or new)

- To define the role of modern methods to support the maintenance of vegetatively propagated *Allium* germplasm—technical abilities, requirements, coordination:
  - virus elimination
  - *in vitro* storage
  - cryopreservation
- To agree on minimum phytosanitary standards and requirements
- To identify gaps in the vegetatively propagated *Allium* collections in a European context and identify needs for collection
- To develop recommendations for presentation to the ECP/GR Steering Committee on the requirements for the development of a system for the long-term conservation and safety-duplication of vegetative alliums in the ECP/GR countries.

Historical overview

Some efforts have been undertaken in the past under the auspices of ECP/GR to manage the vegetatively propagated alliums.

- **In the workplan of the second meeting of the ECP/GR *Allium* Working Group held in Olomouc, Czechoslovakia in 1986**, two centres in Europe were nominated to act as global field genebanks for vegetatively propagated material:
  - the Israeli genebank, in association with the Faculty of Agriculture, Hebrew University of Jerusalem, Israel, for short-day material within 35° latitude; and
  - the Research Institute of Vegetables and Breeding Olomouc, Czechoslovakia, for long-day material, outside the 35° latitudes.

It was recommended that all collection curators forward duplicates of their vegetatively propagated material to the designated field genebanks.

It was agreed that tissue culture of vegetatively propagated accessions and quarantine inspection be an essential function of the designated field genebanks both for incoming and outgoing material.

- **In the fourth meeting of the *Allium* Working Group in Gatersleben, Germany in 1991,** remarkable progress had been made with respect to the passport database development recording the number of vegetative accessions in European collections, whereas difficulties became obvious with respect to the capacity of the field genebanks to maintain such numbers of accessions. Despite the call of the *Allium* Working Group at its 1988 meeting in Wellesbourne, UK, by the 1991 meeting no other institute in Europe had offered to act as an additional field genebank. The Research Institute of Vegetable Crops at Skierniewice, Poland, had provided help in duplicating some accessions. At that time a virus-free *in vitro* collection of garlic was reported to be maintained in the Olomouc institute.

In a critical phase of the existence of the Olomouc institute, during the political reorganization of the Czech Republic, a supporting grant was provided by the UK Ministry of Agriculture, Fisheries and Food to be managed by IPGRI, which enabled the institute to maintain the field collection and repatriate clones to original donor collections. In 1994, a safety-duplicating action was undertaken by the IPK genebank, Gatersleben to transfer 108 accessions of garlic, shallot and other alliums from Olomouc. At that time, it was not possible to maintain *in vitro* cultures at Olomouc.

- **During the fifth meeting of the *Allium* Working Group at Skierniewice, Poland in 1995,** the success of this support action was reported for the long-day collection. The collection in Olomouc had been included into the genebank of the RICP Prague as a

sub-station and, thus, it became part of a stable institution. Some actions were planned for that year to duplicate the shallot collection of the Nordic Gene Bank. In the report on the field collection of short-day *Allium*, it was stated that the vegetative maintenance of *Allium* is rather difficult, and in both collections efforts for rationalization suffer from a lack of funds. In this meeting the first successful activities of the IPK genebank had been reported in establishing a garlic/shallot *in vitro* collection. At the same time, the terminal phase of taxonomic research in the IPK wild species collection was reported. The head of the Taxonomy Department, Peter Hanelt, agreed to establish a reference collection of wild species for research purposes. The Group recommended that researchers using wild *Allium* species should validate their material against the IPK collection and provide sub-samples to IPK after multiplication for subsequent research activities. Other vegetative collections were described from France, the Nordic Gene Bank and Poland.

- **In the sixth meeting of the *Allium* Working Group at Plovdiv, Bulgaria in 1997,** statements were received from both the long-day and the short-day collection curators indicating that their collections had reached their full capacity and further development would depend entirely on additional funds. In the short-day collection, support by the Israeli genebank was very limited and was not sufficient to cover the minimum requirements for preservation of the collection. The *Allium* Working Group recognized the uniqueness of the short-day collection and expressed the wish to obtain further information about the collection. The high scientific value of the two IPK Gatersleben collections was also stressed by the participants. Other vegetative collections were reported from Belgium, Germany, the Nordic Countries, Poland, Russia and Yugoslavia F.R.

- **Since the sixth *Allium* Working Group meeting in 1997:** research on cryopreservation of garlic for long-term storage was started in 1997 at IPK, together with a researcher from the Genebank Skierniewice. Unfortunately, the European Commission did not accept an EU project proposal on cryopreservation of medicinal and aromatic plants including garlic, as was proposed at the sixth Working Group meeting. In the IPK, the research activities on *Allium* taxonomy finished in 1999. Subsequently, it was necessary to negotiate the transfer of the wild species collection from a research status to a genebank collection within the IPK. This process was accompanied with reduction of the collection and reorganization of its maintenance.

In the meantime, numerous activities were running for vegetative alliums within the framework of the EU GEN RES Project. The main partners, CIFA-Córdoba, Spain, and IPK-Gatersleben, Germany, worked on minimum descriptors for characterization, selected a core collection consisting of a "northern" and a "southern" components and established virus-free clones (in the Spanish part, in collaboration with the University of Córdoba). At Gatersleben, a virus-free *in vitro* collection of 95 accessions is in permanent maintenance. The EU GEN RES Project on *Allium* finished in the year 2000 leaving the core collections and the virus-free *in vitro* collection in an endangered state.

Several other collections of vegetative material were established in parallel to the process described here, e.g. a research collection on leek relatives maintained in the PRI Wageningen, the Netherlands.

The process of establishment of vegetative *Allium* collections can be characterized from the onset as being complex and difficult because of the extensive efforts required for maintenance, propagation and phytosanitary measures, which by far outweigh the need to maintain collections based on seed storage. Nevertheless, there has been a permanent quantitative and qualitative growth of the collections in several countries. This growth process is now reaching its limit because of the lack of further funds. This is evident for all

collections to a similar extent. The special status of both the short-day (Israel) and the long-day collections (Olomouc, Czech Republic) have so far not caused any special problem with respect to their safety and support. Measures to rationalize the collections are, therefore, necessary for **all existing *Allium* collections** in a similar way. It is evident that alternative strategies are needed for the future.

The main European collections of vegetatively propagated Allium

The following table gives a preliminary survey on the larger vegetative *Allium* collections (according to the latest available information).

Country	Institute	Species	Approximate no. of accessions	Maintenance methods
Austria	AUTWIEIPP (Institute for Plant Production, Vienna)[4]		30	field?
Belgium	BG Melle[1]	wild species	55	field
Bulgaria	IVC Plovdiv[1]	garlic	140	field
Bulgaria	ESVC Gorna Oryahovitsa[1]	garlic	203	field
Czech Republic	RICP[1]	garlic	600	field
Czech Republic	RICP[1]	shallot	133	field
Czech Republic	RICP[1]	wild species	63	field
France	INRA-Ploudaniel[4]	shallot	80	field
France	INRA-Montfavet[4]	garlic	50	field
France	GEVES-Cavaillon[4]	garlic	40	field
France	GEVES-Brion[4]	shallot	30	field
Germany	IPK[1]	shallot, top onions	300	field, *in vitro*
Germany	IPK[1]	garlic	480	field, *in vitro*, cryo
Germany	IPK[1]	wild species	2000	field
Germany	IPK[1]	leek relatives	40	field
Greece	Greek Genebank[1]	wild species	200	pot culture
Hungary	Tápiószele[1]	garlic, shallot	60	field
Israel	Israeli Genebank[4]	shallot	70	field
Israel	Israeli Genebank[3,4]	garlic	130	field
The Netherlands	PRI[2]	leek complex	500	field, greenhouse
Nordic Countries	NGB[1]	shallot	30	field
Poland	RIVC[1]	garlic	260	field
Poland	RIVC[1]	shallot	70	field
Poland	RIVC[1]	wild species	240	field
Portugal	BPGV[1]	garlic	210	field
Spain	CIFA Córdoba[5]	garlic	640	field
Spain	ESPPOLVAL (Polytechnic University, Valencia)[4]	garlic	73	field ?
Russia	VIR[1]	garlic	325	field (?)
Russia	VIR[1]	perennial wild	330	field (?)
United Kingdom	Kew Gardens	wild	??	??

Sources:
[1] = Sixth report of the *Allium* Working Group
[2] = personal information
[3] = European *Allium* Database 1999
[4] = Fifth report of the *Allium* Working Group
[5] = GEN RES information exchange

The status of the collections is rather diverse, ranging from genebank collections, botanical gardens, research collections, breeding collections, deposits for variety protection to collections within NGOs. Some of them are not entirely sure about future funding. There are also differences in access and availability of samples.

Appendix II. Abbreviations and acronyms

AFLP	Amplified fragment length polymorphism
BPGV	Banco Português de Germoplasma Vegetal, Braga, Portugal
CGN	Centre for Genetic Resources, Wagweningen, The Netherlands
CIFA	Centro de Investigación y Formación Agraria (Agricultural Research and Training Centre), Córdoba, Spain
EADB	European *Allium* Database
ECP/GR	European Cooperative Programme for Crop Genetic Resources Networks
ELISA	Enzyme linked immunosorbent assay
ESVC	Experimental Station for Vegetable Crops , Gorna Oryahovitsa, Bulgaria
GCLV	Garlic common latent virus
GDV	Garlic dwarf reovirus
GEVES	Groupe d'étude et de contrôle des variétés et des semences (Varieties and Seeds Study and Control Group), France
GRU	Genetic Resources Unit (of HRI)
HRI	Horticulture Research International, Wellesbourne, United Kingdom
IGB	Israeli Gene Bank for Agricultural Crops, Bet-Dagan, Israel
INIA	Instituto de Investigación Agraria (Agricultural Research Institute), Spain
INRA	Institut national de la recherche agronomique (National Agronomic Research Institute), France
IPGR	Institute of Plant Genetic Resources, Sadovo, Bulgaria
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany
IVC	Institute of Vegetable Crops "Maritsa", Plovdiv, Bulgaria
LYSV	Leek yellow stripe virus
N.AG.RE.F	National Agricultural Research Foundation, Greece
NGB	Nordic Gene Bank, Alnarp, Sweden
NGO	Non-governmental organization
OYDV	Onion yellow dwarf potyvirus
PCR	Polymerase chain reaction
PRI	Plant Research International, Wageningen, The Netherlands
RAPD	Random amplified polymorphic DNA
RICP	Research Institute for Crop Production, Prague, Czech Republic
RIVC	Research Institute of Vegetable Crops, Skierniewice, Poland
RIVGB	Research Institute of Vegetables Growing and Breeding, Olomouc, Czech Republic
SLV	Shallot latent virus
SYSV	Shallot yellow stripe virus
TPIA	Tissue print immunosorbent assay
UPOV	Union internationale pour la protection des obtentions végétales (International Union for the Protection of New Varieties of Plants), Geneva, Switzerland
VIR	Vavilov Institute of Plant Industry, St. Petersburg, Russian Federation

Appendix III. Agenda

Ad hoc *meeting on the European collections of vegetatively propagated* Allium *Gatersleben, Germany, 21-22 May 2001*

Sunday 20 May 2001

Visit to the Harz mountains

Monday 21 May 2001

9.00 **Welcome address** (*Prof. Dr A. Graner, Head of the Genebank*)
9.15 **Introduction** (*J. Keller, L. Maggioni and D. Astley*)
9.40 - 10.30 **Reports and recommendations/proposals from the participants** *(Czech Republic, Israel, NGB)*

10.30 - 11.00 *Coffee break*

11.30 - 12.30 **Reports and recommendations/proposals continued** *(Germany, Poland, France, Portugal)*

12.30 - 14.00 *Lunch*

14.00 - 15.30 **Reports and recommendations/proposals continued** *(United Kingdom, Netherlands, Spain, Bulgaria, Greece)*

15.30 - 16.00 *Coffee break*

16.00 - 17.30 **Discussion and recommendations on**
- Minimum phytosanitary standards (*introduced by A. Senula*)
- Measures for safety-duplication and coordination in the ECP / GR countries

20.00 *Social dinner*

Tuesday 22 May 2001

9.00 - 10.30 **Documentation issues**
- Survey on passport data and other information (*D. Astley*)
- Documentation of vegetatively propagated alliums at IPK (*H. Knüpffer*)

10.30 - 11.00 *Coffee break*

11.00 – 12.30 *Visit to the* Allium *genebank*

12.30 - 14.00 *Lunch*

14.00 - 15.30 **Modern methods for conservation**
- Experience on *in vitro* storage and cryopreservation at IPK, Germany (*J. Keller*)
- Cryopreservation at RICP, Czech Republic (*J. Zamecnik*)
- Discussion and recommendations

15.30 - 16.00 *Coffee break*

16.00 – 18.00 **Network proposal and conclusions**

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