

Report of a Working Group on Allium

Eighth Meeting of the ECPGR *Allium* Working Group 11-12 October 2022, Skierniewice, Poland



The **European Cooperative Programme for Plant Genetic Resources (ECPGR)** is a collaborative programme among most European countries aimed at rationally and effectively conserving *ex situ* and *in situ* plant genetic resources for food and agriculture, providing access and increasing their sustainable use (<u>http://www.ecpgr.cgiar.org</u>).

The Programme, which is entirely financed by the member countries, is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries. The Coordinating Secretariat is hosted by the Alliance of Bioversity International and CIAT.

The Programme operates through Working Groups composed of pools of experts nominated by the National Coordinators. The ECPGR Working Groups deal with either crops or general themes related to plant genetic resources (crop wild relatives, cryopreservation, documentation and information, and on-farm conservation). Members of the Working Groups carry out activities based on specific ECPGR objectives, using ECPGR funds and/or their own resources.

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Cover photos: *Allium neapolitanum* Cirillo, Rome, Italy. Credit: L. Maggioni; Group photo of the participants in the *Allium* Working Group meeting in Skierniewice, Poland, 11-12 October 2022. Credit: InHort.

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SUMMARY REPORT OF THE MEETING

Introduction

Considering the opportunity offered by the ECPGR mode of operation during its Phase X (2019–2023), the Working Group (WG) on *Allium* submitted in January 2020 a request to organize its Eighth WG meeting. The objectives of the meeting were to promote collaborative activities, develop new projects, discuss problems and their solutions for germplasm preservation, and exchange information and experiences. Participants were selected based on a their expression of interest to join the meeting. The location in Poland was selected based on a kind offer made by the National Institute for Horticultural Research (InHort), Skierniewice and the fact that the current non-membership of Poland to ECPGR would not allow funding the travel costs of Polish curators to a different country. In March 2020, the ECPGR Executive Committee (ExCo) approved the meeting, which was initially scheduled to take place in May 2020. However, the COVID-19 pandemic made it not possible for people to travel and meet safely for a long time. Eventually, the conditions for organizing a meeting in person improved and it was possible to come together in Skierniewice for the Eighth *Allium* WG meeting in October 2022.

Opening of the meeting

The Chair of the Working Group, Helena Stavělíková, welcomed the group to the Eighth meeting and asked everyone to briefly self-introduce.

The Director of InHort, Dorota Konopacka, welcomed the group to the institute and thanked for the decision to hold this meeting in Poland, even though the country is not an ECPGR member. She expressed eagerness to participate in collaborative work, considering the richness of genetic resources in Poland. Skierniewice is considered the Polish capital of horticultural science. Its climate is characterized by hot summers and cold winters, but these have become less severe, with less snow than in the past, which is a problem for fighting insects.

The institute was established in 1951. It is supervised and funded by the Ministry of Agriculture and Rural Development and it is also monitored by the Ministry of Science and Education. Its ten research departments cover all aspects of horticultural production, benefiting from 165ha of experimental fields, 2ha of greenhouses, 33 laboratories and 130 research staff. It bears the tasks of developing new technologies, but also improving production per se.

The Director of the InHort Department of cultivars testing, nursery and genetic resources, Mirosłav Sitarek, presented the activities of the department. The systematic collection, evaluation and conservation of fruit plant cultivars in Poland started in 1921, when the Department of Fruit Growing at the Agricultural University of Warsaw was established in Skierniewice. Presently, field collections of cultivars of fruit trees cover over 3,000 accessions, of which more than 1,300 apple accessions. Additionally, over 1,000 accessions are berry cultivars. InHort takes care of mostly field collections of various species of fruit plants, but also vegetables, and medicinal and ornamental plants. A specific section is dedicated to cryopreservation of garlic. Since 1981, also seeds of more than 10,000 accessions of 50 species of vegetable plants have been collected and stored. The institute's tasks include regeneration and valorization, in cooperation with seven partner institutes across Poland.

Information on the ECPGR Programme

(L. Maggioni)

The ECPGR Secretary summarized objectives, structure and mode of operation of ECPGR. The expectations from the activities of a Working Group and the opportunities to receive funding were also explained. Key features of the ECPGR pillar initiatives, AEGIS, EURISCO and EVA, were outlined. The group was also invited to consider submitting papers to the *Genetic Resources* Journal, which is managed by the ECPGR Secretraiat and is offering free publishing of peer-reviewed articles and free reading access. Recent and ongoing EC-funded Horizon projects with direct involvement of the Secretariat were mentioned, with emphasis on the 'Plant Genetic Resources Strategy for Europe', a recent outcome of the 'GenRes Bridge' project. Finally, the WG was advised about the expected continuation of ECPGR into Phase XI (2024–2028), with detailed decisions about priorities and mode of operation expected to be finalized at the next ECPGR Steering Committee meeting in May/June 2023.

In reply to a few questions from the audience, L. Maggioni gave indications on the possible way for the WG to get involved in EVA, starting with a dialogue with breeding companies and verification of their interest to get involved in the collaborative evaluation of genebank accessions in multi-location trials. He also confirmed that the possibility to organize hybrid ECPGR meetings (in person and online) will likely become a regular occurrence. Finally, he confirmed that prioritization and sharing of genebank accessions, activities and operations across Europe will remain a key objective of ECPGR in the near future.

Report on the work of the Working Group over the past years

(H. Stavelikova)

The Allium Working Group was established in 1982 during the first Phase of ECPGR.

Twelve meetings were organized during its 40 years of existence – seven regular, two ad hoc and three meetings via the Vegetables Network (VEGNET). The last regular meeting took place in 2011, in Thessaloniki, Greece. The WG was chaired by three Chairs during its history (Dave Astely from 1982–2011, Joachim Keller from 2012–2014 and Helena Stavelikova from 2015 until present. Currently, the WG has 64 members/observers from 34 countries.

Milestones reached by the WG since the last meeting include:

- 2013 upgrade of the European *Allium* database to become searcheable online (by Markus Oppermann, IPK, Gatersleben)
- 2015 Agreement on crop-specific standards for field genebanks
- 2016 Grant Scheme Activity 'Safeguarding of potato onion (*Allium cepa* L. Aggregatum group) and garlic (*Allium sativum* L.) crop diversity in North Europe Baltic region (SafeAlliDiv)' was approved for funding by ECPGR
- 2017 Agreement on crop-specific genebank standards for *in vitro* culture and cryopreservation
- 2017 First SafeAlliDiv meeting in Tallin, Estonia
- 2018 Second SafeAlliDiv meeting in Olomouc, Czech Republic
- 2020 Final report of SafeAlliDiv published online
- 2020 SafeAlliDiv article published in *Genetic Resources and Crop Evolution*: Genetic characterization of European potato onion (*Allium cepa* var. Aggregatum G. Don)
- 2022 Eighth meeting in Skierniewice, Poland.

The European *Allium* database was established in 1984 by the ECPGR *Allium* WG and originally maintained by Horticulture Research International, Wellesbourne, UK and then by the University of Warwick, UK. It was last updated in 2007 as part of the EU GENRES EURALLIVEG project. Since 2012, it has been maintained by the Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben (IPK), Germany. The database contains 14,194 accessions representing 550 species from 43 institutions in 29 countries.

Information provided to EURISCO about the genus *Allium* relates to 14,021 accessions and 412 taxa from 32 countries. Of these, 1248 accessions are registered as part of AEGIS.

Results of the SafeAlliDiv project

L. Lepse

This Activity, funded under the ECPGR Grant Scheme, Fourth Call of Phase IX, was carried out between 2017 and 2020 by partners from Croatia, the Czech Republic, Estonia, Finland, Latvia, Lithuania, Norway and Sweden. The aim was to reach a better understanding of the genetic diversity and to find duplications in collections of potato onions from partner countries, also complementing molecular marker analysis of Nordic potato onions that had been carried out earlier on in Sweden and Finland.

Eleven markers were used to analyze 264 accessions. The samples clustered rather regularly, based on their country of origin, with Croatian and Lithuanian samples clustering separately from each other and from the rest of the collection. It was concluded that accessions in the collections are unique, with unique alleles identified almost in each country. The value of the material was confirmed, as well as the importance of conserving large numbers of accessions and further collecting material. In fact, the majority of accessions are genetically unique and thus it is possible that not all the genetic diversity has yet been captured in the collections.

The results have been published in: Rungis *et al.* 2020. Genetic characterization of European potato onion (*Allium cepa* var Aggregatum G. Don) collections. *Genetic Resources and Crop Evolution* 68: 657-665. <u>https://link.springer.com/article/10.1007/s10722-020-01014-2</u>.

Report on national Allium collections

Czech Republic

H. Stavělíková

Eight collections in the Czech Republic conserve 903 *Allium* accessions (605 *A. sativum*, 132 *A. ascalonicum*, etc.). It is mainly vegetatively propagated accessions (777), with some seed accessions (54) and 177 under cryopreservation.

The overall rate of safety duplication of the collection is 42%, with duplications of vegetatively propagated material conserved at IPK, Germany and RIPP, Slovakia and a few accessions duplicated as seed in the Svalbard Seed Vault. The Czech genebank also accepted safety duplicates from a few countries.

A large part of the garlic collection originates from the former Soviet Union and Czechoslovakia. The biological status includes current varieties and breeding material from the Czech Republic and Slovakia; landraces from South Moravia and the Carpathian area, collected in the 1980s and 1990s; wild and primitive forms of garlic from Central Asia and Siberia, collected in 1988 and 1990.

ECPGR and national descriptors are used for documentation. The documentation system is GRIN-Czech. Characterization has been carried out at 99% level for garlic, 100% for shallot and onion, 80% for spring onion; for leek and chive, the material needs to be multiplied and then characterization will be started. All data, including C&E data, are refreshed every year in EURISCO. Pictures are also available.

For the acquisition of new material, good cooperation is ongoing with other genebanks (for repatriation), seed companies, breeding companies and local growers.

For the use of the collection, 90% of the accessions is available with SMTA. Good cooperation is established with universities, breeding companies and the exhibition centre Flora Olomouc. A research project based on molecular analysis is ongoing to improve garlic yield and quality through selection for drought and cold-resistant clones.

The current main problem faced is climate change, as it is becoming necessary to transplant garlic later, in early December and the growing season is shorter. Also, spring starts later and shallot can go to the field in April, compared to February or March a few years ago.

Proposals for the WG activities include safety duplication, project preparation, cooperation with other WGs and increased communication with members of the Group.

Estonia

P. Põldma

Collecting missions of *Allium* bulbs and seeds for conservation and research were carried out in 2014 from villages in the Peipsi lake area. Field experiments and lab analyses were then performed in 2014 and 2015. The Nordic-Baltic documentation system (<u>https://www.nordicbaltic-genebanks.org/gringlobal/search.aspx</u>) includes information only on seed-conserved material (i.e. only potato onion Jogeva3, selected after 13 years from material collected in 1942). The collection in the field at Jõgeva includes one accession of garlic repatriated from Denmark and four landraces of potato onion, including one accession from Pirisard island, imported from Russia, and the 100-year-old Sangaste onion. The Department of Horticulture of the Estonian University of Life Sciences, Tartu, conserves a garlic cultivar collection, with commercial material from France, Lithuania, Poland and Ukraine.

Everyone is invited to the garlic festival in Kuremaa, which takes place every year on 20 August, also with exhibitions and competitions for bulb size.

Finland

T. Suojala-Ahlfors

The Natural Resources Institute Finland (Luke), has the duty to conserve Finnish material in field collection. The *Allium* collection mainly consists of potato onion (34 accessions, 29 of which are landraces), which generates 3 to 15 bulbs from one at harvest time in autumn. One accession is preserved in cryo and there will be more in the future. There are also some tree onion accessions (10), a few garlic (7) and one sand leek (*A. scorodoprasum*). Safety duplication of part of the potato onion collection (41%), is supported by the agro-environmental programme with schools, societies, institutions and private persons conserving the material in other sites than Luke. Most material conserved is Finnish, with one potato onion accession from Estonia.

The ECPGR Descriptors are used for documentation. Potato onions are 90% characterized, but no C&E data have been delivered to EURISCO. Discussion is ongoing on whether to collect garlic, which has not been grown for a long time in Finland. The acquisition strategy relies on

citizens alerted through the media. Most of the material is available to users with SMTA. An ongoing project 'Onions to use – promoting the cultivation of potato onion (2020–2022)' aims at producing virus-free propagation material and enhancing the knowledge and use of traditional potato onions.

The main problems encountered are the cost of maintaining field collections and virus diseases. Additionally, potato onions in backup collections are not always growing very well and there are problems with winter storage. *In vitro* culture of onion and developing cryopreservation methods are also time-consuming, thus progress in this respect has been very slow.

Germany

M. Nagel

Two major collections in Germany, the *Allium* Crop Collection (1,400 accessions of 76 species) and the Taxonomic Reference Collection (ca. 1,300 accessions of 287 species) have been merged into one genebank collection, which is permanently maintained in the field, replanted every 5-6 years. However, the Taxonomic Reference Collection is facing troubles with the retirement of taxonomists and specific gardeners. The budget is reduced for this collection and decisions need to be made to reduce the costs, therefore part of the collection might be at risk. It would also not be possible to put everything under cryo, which takes time. In addition, conservation as seed would not be ideal, since open pollination has created mixed genotypes.

In total, 1,000 genebank accessions are kept in the field every year. Both the garlic and onion collections include material from all over Europe, with a prevalence of landraces, but also improved cultivars and breeding material. Overall, 20% of the collection is backed up under cryo or as seed at IPK. The documentation system is GBIS, and the ECPGR Descriptors are used. The collection is not characterized, apart from the '*Allium* and Shallot Core Collection' and these data have been provided to EURISCO.

There is no intention to acquire more samples, due to the existing problems. The material is 90% available through SMTA. There is no ongoing cooperation with users but an ongoing sequencing of garlic and shallot with phylogenetic trees was developed, and a proposal on 'Garlic metabolite analysis with organic farmers' was submitted. The main problems are the management and budget of the Taxonomic Reference Collection, the lack of *Allium* trained taxonomists due to retirement, the lack of cryopreservation protocols for different *Allium* species, the lack of funds to support cryopreservation, the identification of duplicates and the definition of respective threshold levels, and the management of different databases.

The challenges with cryopreservation consist in the lack of material of good quality to cryopreserve (at the moment 159 accessions are under cryo), even though it is clear that the collection cannot be maintained *in vitro* and the focus remains on moving from field to cryo.

Proposals for the Working Group activities include characterization, evaluation and genotyping to support: the identification of duplicates and unique material; taxonomic decisions; the exchange of material for collection management (and gaps filling); usability of material by breeders; prioritization of cryopreservation of European accessions; guidance for collecting missions. Criteria need to be defined for duplicate identification and the databases require rationalizing and improvement. A cryopreservation workshop could be organized. Support is sought for the maintenance of the large wild *Allium* collection.

Greece

C. M. Cook

The main collections in Greece contain 347 *Allium* accessions, mainly wild alliums (113), onion (106), leek (87) and garlic (41). Several species of wild alliums were collected by Dutch researchers, were regenerated and seed was sent back to Greece. There is a high diversity in morphology and tastes, with each community growing for local consumption. No safety duplicates exist, except for wild alliums in the Netherlands. The most important collection, common onion, consists of only Greek accessions, all traditional cultivars and landraces.

Morphological characterization, according to the ECPGR Descriptors, was completed for 62% of the accessions. These are documented in Microsoft Excel spreadsheets and the C&E data have not been delivered to EURISCO. Pictures are available, but not online. Updated data of the collection were not forwarded to EURISCO since the year 2000 and the decision about this step has remained pending at the ministerial level.

It is considered desirable to fill gaps in the collection, but collecting missions are dependent on funding and staff availability. The acquisition strategy involves direct collection of traditional cultivars (landraces) from farmers and of crop wild relatives from different geographical regions of Greece. Samples are deposited in the Greek Genebank. Presently, the garlic collection is being updated with new accessions obtained from different regions of Greece.

Availability of the material is not possible through SMTA. Specific licences must be released from the Ministry of Rural Development and Food. Small seed samples are available for farmers in the locations of seed acquisition/collection and to NGOs. Small seed samples are also made available for research purposes.

The main problems encountered are staffing (particularly support staff) and limited funding which affect primarily the ability to regenerate stored seed samples, the maintenance of live field collections and collecting expeditions. Heavy bureaucracy is also a major problem since the Greek Genebank must have the permission of the Ministry of Rural Development and Food to distribute seeds, and update and upload information in databases.

Italy

A. Natalini

Collections reported in Italy include 140 *Allium* accessions, mainly garlic (73) and onion (61), mostly traditional cultivars/landraces of Italian origin. Safety duplication is covered for about 5% of the accessions. Part of this collection is characterized and data have partly been delivered to EURISCO. For documentation, MCPD descriptors and MS Excel files are used. There is no clearly defined acquisition strategy at the national level. The material is generally not available, except for a few accessions conserved in the genebank in Udine, which are available under SMTA. The main problems are the lack of funds and technical personnel for field operation, maintenance and safety duplication. The risk to lose germplasm is very high (for example, 48 unique garlic accessions conserved at the University of Basilicata were lost during the COVID pandemic). Another problem is the fragmentation of the collections, which are split among several institutions. Proposals for collaboration within the WG are directed at collecting, characterization and breeding.

Latvia

L. Lepse

The collection includes 142 Allium accessions, mainly garlic (92) and potato onion (49) landraces collected during collecting missions, since people are still growing local garlic and potato onions. The large majority of the garlic accessions are bolting. Both onion and garlic collections are safety duplicated in two campuses of the Institute of Horticulture (at Dobele and Pūre). The documentation system consists of institutional MS Excel files. Almost 100% of the collection is characterized and C&E data are delivered to EURISCO, although they require updating. Pictures of bulbs and leaves are regularly taken. The acquisition strategy foresees the establishment of a collection of chives (A. schoneoprasum) and wild garlic (A. ursinum) which are present in nature. It could be useful to continue collecting missions, especially in the case of potato onion, since its cultivation is reducing, while garlic is not so endangered. All the material is available under MTA. Cooperation is ongoing with farmers for variety registration of their clones; producers are interested in the processing of potato onion, due to its high pungency. Ongoing projects involve the identification, collection and research of the genetic potential of *in situ* cultivated plants for food and agriculture and their wild relatives (national) and a farmer-initiated variety registration for two clones (private). The main problem is that costs are rising for field collection maintenance and national funds are limited. Collecting missions for wild garlic are necessary. Cryopreservation could be a useful collaborative activity.

The Netherlands

R. Lievers

The main part of the Dutch *Allium* collection consists of onion seeds (228 accessions). There is no cryoconservation or field conservation. Other smaller collections are those of leek (92), spring onions from Japan (45), chives (25) and wild *Allium* species (45). The collection has remained stable in the past years, but there are now plans to expand. The entire collection is duplicated in Warwick, UK, and is partly triplicated at the Svalbard Seed Vault. Many accessions are from the Netherlands, and a few from other countries, derived from various collecting missions.

Most material is improved cultivars, but there are also a good number of landraces. The 107 wild leek accessions collected in Greece in 2009 are still in multiplication and not available through SMTA, therefore not part of the regular collection. Some accessions were lost since they failed to multiply.

For documentation, 15–20 traits are used, partly derived from ECPGR and UPOV descriptors. An Oracle documentation system is used. Accessions are 90% characterized and data uploaded to EURISCO.

The plans to expand the collection are ambitious. The government acknowledged that the overall CGN collection needs to expand, therefore the aim is to double the current size and reach 45,000 accessions to be stored in a new genebank to be built. Consequently, also the *Allium* collection will expand. A recent multi-crop collecting mission to Albania was intended to acquire modern varieties from breeding companies, as well as exchange and repatriate material from other genebanks. All material in the regular collection is available through SMTA. Cooperation is regularly ongoing with breeders, NGOs, farmers and with breeding companies that regenerate the collection in kind and also evaluate the material.

The main problem is multiplication since it is expensive and takes time. An unexpected problem was obtaining material (hybrids) from breeding companies, even if no longer in the market. Another important challenge is the organization of missions to centres of diversity. An ongoing project is about the evaluation of wild leek from Greece by five breeding companies for *Alternaria porri*, *Phytophthora porri*, *Thrips tabaci* and *Puccinia porri*.

Proposals for collaborative work relate to joint regeneration and collecting missions.

Norway

I. M. Vågen

All seed collections are deposited at NordGen, while the conservation of Norwegian vegetative material is the responsibility of the national programme, kept in clonal archives or field collections. Two clonal field genebanks for shallot and wild garlic are established in different locations. The shallot collection was part of the 'SafeAlliDiv' collection and some genetic analysis was carried out to identify possible duplicates or unique accessions, according to the markers used. A large number of possible duplicates was found in this collection. Verification of the list of duplicates made in the field indicated that a lot of them are very similar, but others listed as duplicates are very different.

The collection is quite fractionated in institutions linked to the national resource centre, or at museums. The tiny quantities are not in the condition to be shared. At the Norwegian Institute of Bioeconomy Research (NIBIO), there are some chives, one leek cultivar and some spring onions. All seed accessions are safety duplicated in Svalbard. Around half of the shallot accessions are also safety duplicated in the Czech Republic. Accessions from various clonal archives and museums are mostly not safety duplicated. Thirty percent of the seed accessions are characterized with ECPGR morphological descriptors and available C&E data are delivered to EURISCO. There is no active acquisition of new material, although it would be useful to look for material that is being lost. The institute is open to accepting material offered by donors. NordGen accessions are available via SMTA. Vegetative material is shared through a seed saver/hobby grower organization 'Kvann' if there is enough material to distribute. The main problems are the virus infection of the shallot collection and the lack of funding for preservation activities. For collaboration, it is proposed to better link conservation with use. Involvement in the EVA Network would also be very interesting.

Poland

M. Chojnowski

The Polish *Allium* collecting activities started in 1980, with material obtained mostly from Poland, but also Ukraine, Lithuania, Russia, etc. Former curator Teresa Kotlinska collected a huge amount of accessions. After 2018, two new curators at InHort, Skierniewice, took up the challenge of maintaining collections in the field, including 360 winter garlic, 277 spring garlic, 281 shallot and 104 perennial accessions. Onions (430) and leek (63) are deposited as seeds in Radzikow. The material of the overall *Allium* Polish collection is primarily landraces (1,300). The Cryo genebank at InHort conserves 227 garlic accessions. Polish accessions are safety duplicated under cryo at IPK, Germany and CRI, Czech Republic. Each year ten new accessions are added to the cryopreservation collection. Information is kept in MS Excel files, but is being transferred to the GRIN Global system, which is used for genebank management.

Portugal

I. Gomes da Silva

The Portuguese Plant Germplasm Bank in Braga conserves material obtained from more than 130 collecting missions. Among these, were international collecting missions for vegetables, undertaken between 1990 and 1994 by Dave Astley, Horticulture Research Institute, UK, by Takeomi Etoh, University of Kagoshima, Japan and later by Rena Farias.

Allium sativum and *A. cepa* are the most represented species with 288 and 219 accessions, respectively. Garlic is maintained in field, cryo and *in vitro*; onion is maintained *ex situ* as seed. Also, other *Allium* species are present in the collection.

Safety duplication is almost complete for garlic (99%), and in progress for onion (30%) and leek (7%). The large majority of accessions are landraces from Portugal. GRIN Global is used as a documentation system. Since 2011, the characterization was based on the ECPGR list of descriptors published in 2001. The collection is largely characterized morphologically, molecularly and chemically, but no C&E data have been transferred to EURISCO yet. The acquisition strategy depends on project funding and is based on collecting missions of traditional cultivar/landrace and/or crop wild relatives, exchanges between genebanks and offers from farmers. The material is available in limited quantities, with SMTA.

The main problem, in the field collections, is climate change, which is affecting the good development of the *Allium* crops. In addition, productivity is decreasing with the ageing of the material in the field and *in vitro*, while seed viability during cold storage is lost very quickly.

Slovenia

J. Šuštar Vozlič

The collection at the Agricultural Institute of Slovenia includes mainly accessions from Slovenia and other countries from ex-Jugoslavia. In total, there are 62 onion, 43 garlic and 21 wild *Allium* accessions. Most important onion and garlic accessions will be identified and included in cryo. Garlic and onion are all landraces. Safety duplication has not been implemented. For documentation, UPOV and ECPGR descriptors are used. Data are maintained in MS Excel and Access files, since the country decided not to use GRIN Global. About 50% of the material has been characterized, but not included in EURISCO.

After genetic analysis of garlic will be completed, transfer to *in vitro* conditions for storage will start with the most important accessions first. The acquisition strategy includes direct collecting and exchanges from companies and genebanks. In certain parts of Slovenia, some local accessions of onion, garlic and shallot may still be found. The presence of accessions from Slovenia in foreign genebanks will be checked and in case, the process of repatriation will start. Fifty percent of the material is available under SMTA. A project of characterization of garlic and onion accessions under the Rural Development Programme 2014–2020 is ongoing.

The most urgent things to do are the transfer of the garlic collection to *in vitro* culture (or cryopreservation) and the safety duplication.

It is proposed to the group to focus on crop wild relatives and on-farm conservation.

Spain

M. de los Mozos Pascual

Nine different institutions with different priorities and missions are preserving *Allium* material. The most important collection is the common onion, with 1,022 accessions (not all available, since regeneration is pending). Garlic and leek are significant collections with 471

and 202 accessions, respectively. Another important collection is elephant garlic (*A. ampeloprasum*) with 69 accessions, including not only wild samples but also cultivated material from the Canary Islands. Overall, the Spanish collection includes 1,288 accessions conserved as seed, while mainly garlic and elephant garlic are kept in the field. Nothing is preserved *in vitro* or cryo. Onions, garlic, leek and elephant garlic are all mainly landraces from Spain with a few accessions also from other countries, and many duplicates from other genebanks.

There is also an important part (120 accessions) of the collection of unknown origin. Safety duplication is partly established for onion (55%) and leek (62%), but none for garlic. For documentation, MS Excel and Access files are used. The collection is 30% characterized and 61% of these data are entered into EURISCO. There are no specific plans at national level to fill gaps in the collection. The coordination among the different Spanish genebanks involved with *Allium* is a priority. The acquisition strategy mainly consists in collecting missions; exchanges with genebanks are also important. Approximately 70% of the material is available with SMTA, otherwise with a specific MTA, which is simplified for direct use by farmers and other small users.

Institutions offer advice on the possible use of *Allium* genetic resources to researchers, farmers, agricultural associations, schools, urban gardeners, etc. The recovery and enhancement of traditional crops in different parts of Spain can be carried out in a participatory way. Farmers occasionally collaborate for the regeneration of the *Allium* collections.

The main problems are institutional instability, with fragmented responsibilities and dispersed material, as well as high administrative obstacles to obtaining funds to maintain the collection. No funds were allocated during the last years from the National Programmes. The risk to lose garlic accessions due to phytosanitary problems is high.

It is proposed to the group to help retrieve lost accessions in the Spanish garlic collection and to create safety duplicates for the most representative materials.

Cryopreservation – State of the art for vegetatively propagated Alliums

(M. Faltus, Co-Chair of the Cryopreservation WG)

The plant physiology and cryobiology team at CRI, Prague, was presented, as well as the team and cryobank milestones, including 45 years of cryobiology study and 20 years of cryobank operation. The strategy of cryoconservation in the Czech Republic involves safety duplication of the basic collections with different storage methods and localities, keeping in cryo the most valuable genetic material of Czech origin. The Central cryobank in CRI Prague receives from curators across the country, often from private companies, the most valuable samples for their backup. A total of 502 accessions from all species (fruits, garlic, potato and hop) are currently cryopreserved.

Cryopreservation is a method for the long-term storage of living organisms, or their parts, at ultra-low temperatures. It is based on the principle that material is conserved in a glassy state through previous massive dehydration. Two basic approaches can be implemented for dehydration and way of cooling: 1) Freezing dehydration, which is a controlled ice crystal growth; 2) Vitrification, which avoids ice crystal growth either through air-dehydration (encapsulation-dehydration) or osmotic dehydration (vitrification method).

In the case of garlic and shallot, different starting materials can be used, with different approaches and also different levels of efficiency. Eventually, the cryobank in Prague decided to adopt a DMSO-free methodology.

After many years of international collaboration through EC and ECPGR-funded projects, a

new ECPGR Working Group on Cryopreservation was established this year. Current issues to be addressed are that there is no extensive and reliable cryobank infrastructure in the EU, backups of crop collections are not coordinated and cryopreservation methods are not always available for routine use. Personnel with specific skills are required, considering that transfer of cryoprotocols is not easy. Possible objectives of this Working Group are the establishment of long-term storage facilities and capacity building of experienced cryopreservation researchers; encouraging advanced plant cryopreservation research in Europe; exploring available cryopreservation technologies for germplasm of species that produce non-orthodox seeds; considering the possibility of cryopreserving orthodox seed collections (short-lived seeds as leeks, onions and parsley); extending cryopreservation to crop wild relatives or wild species and trees; increasing collaboration between European scientists and institutes holding crop collections; and improving new biobank technologies and data management systems for cryopreserved collections.

The main goals of the Working Group will be: to increase networking aiming to involve relevant stakeholders in better organizing and implementing cryopreservation in Europe; to enhance research improvement with standardized methods and protocols easily applicable in the genebanks; to define a strategy of conservation about methodologies, facilities and priority species/genotypes.

In the following discussion, it was clarified that it is important that no genetic change takes place during the garlic cryoconservation; there is currently a debate about the occurrence of epigenetic changes. Genetic changes might occur if the regeneration is carried out through callus, which should be avoided. The occurrence of varieties that are recalcitrant to regeneration needs to be overcome with further research. It was reiterated that a strategy is needed in Europe, for example, to define whether it would be useful to centralize cryoconservation and define the priority samples that should be cryopreserved.

Overview of the global Allium collections

C. Kik

A global overview of *Allium* genetic resources, including between and within species, genebanks, and regions, is important to enabling informed decisions about the enlargement/rationalization of the genebank collections, planning collecting missions, etc.

The proper identification of botanical names is also important (see the Nomenclator Allioruma world guide of *Allium* names and synonyms, published in 1998 by Gregory et al.), as well as having good *Allium* descriptors for passport and C&E data (see the IPGRI/ECPGR/AVRDC *Allium* descriptors published in 2001).

For historical aspects of global *Allium* resources the first reference is *Genetic Resources of* Allium *species*, IBPGR publication of 1982, prepared by Astley, Innes and van der Meer. They identified > 9,000 accessions and onion landraces as priorities for collecting. A book chapter by Astley (1990) on 'Conservation of Genetic Resources' in the book *Onions and Allied Crops* by Rabinowitch & Brewster eds., is a text on genebank management procedures, from collecting to utilization. The chapter by Keller & Kik (2018) on '*Allium* genetic resources' in *The* Allium *genomes* by Shigyo et al. eds., is an updated text of the 1990 chapter, comprising all aspects of genebank management and a number of related issues.

Important online global crop portals have dramatically improved access to documentation of genetic resources. The most important for information on genebanks is Genesys (<u>www.genesys-pgr.org</u>) and for botanic gardens is PLANTSEARCH

(<u>https://tools.bgci.org.plant_search.php</u>). A specific *Allium* crop portal is the European *Allium* database (<u>https://ealldb</u>.ipk-gatersleben.de/EALLDB/faces/home.jspx) maintained by IPK at Gatersleben, Germany.

Currently, the total number of *Allium* accessions recorded in Genesys is 20,627 and 6,270 in PLANTSEARCH. With respect to the existing 1,038 *Allium* species, 56% are represented either in genebanks or botanical gardens. Therefore, large gaps still exist, if we wished to collect all species, but this remains a strategic question. Most accessions represent cultivated species, not wild species. Accessions are conserved in 86 countries, with the largest collections in the USA, Germany, Spain, Russia and the UK. However, information on accessions conserved in Russia is currently limited to 14 accessions in Genesys. The number of *Allium* accessions per biological status of the material held in genebanks worldwide, based on Genesys, includes > 7,000 landraces, ca. 5,000 improved cultivars and ca. 3,500 wild species. No GMO accessions are conserved in genebanks, although these were developed for garlic and shallot, with insect resistance. The method of conservation of *Allium* accessions are in cryo (340) and *in vitro* (136). Nearly 20,000 seed accessions are safety duplicated at the Svalbard Seed Vault and ca. 18,500 at 16 different sites. Ca. 2,000 accessions are expressely indicated as 'available for access'.

The vegetative *Allium* field collection in Olomouc is duplicated at IPK and 200 garlic accessions are maintained in cryo in a trilateral safety duplication organized by the Czech Republic, Germany and Poland. The duplication between genebanks, analyzed in EURISCO, is variable, with a mean of 47%, which is probably too high in terms of a rational and integrated conservation system.

In conclusion, the development of crop portals has greatly improved the knowledge of the content of global *Allium* genetic resources; a new conservation technique (cryoconservation) has been developed for *Allium*; almost all *Allium* accessions have been safety duplicated; large gaps still exist in global *Allium* collections, both within and between species; and the need for *Allium* collecting missions is still present, however current international and/or national regulations for access are often not helpful in this respect.

The European Allium Database (status and perspectives)

(Discussion introduced by M. Nagel)

The European *Allium* Database was transferred in 2012 by Dave Astley from Warwick, UK, to Joachim Keller, IPK, Germany. However, the database was not updated further. It mostly contains passport data. It is suggested to verify whether the data are all available from EURISCO and whether any missing data could be transferred, although images cannot be transferred. It would be important in any case not to throw away the database.

The breeder's perspective (main needs and expectations from genetic resources)

(L. Nedorost, MoravoSeed, Czech Republic)

MoravoSeed is a breeding company since 1991 and registered 420 vegetable varieties so far, including onion, leek, garlic, shallot, etc. Vegetables are multiplied in fields all over the world. Two breeding stations are located in the Czech Republic, at Svijanský Újezd in the north and at Lednice in the south, where the headquarters are also based.

Garlic breeding is demanding. About 20 years are required to obtain improved cultivars, since there are no seeds produced. Variability is very low and variation is obtained through the use

of mutagens, such as colchicine. The new CRISPR approach is now being introduced. The first registered garlic cultivar was Ducat, in 1995, now the most grown garlic in the Czech Republic, as winter type. Unikat was registered in 2008, 20 years after, and after another 12 years, Topaz was registered in 2020 (a winter type hard neck). The new soft neck NŠL – GOL will be registered soon.

The shallot cultivar Schelia, reproduced by seed, was registered in 2019.

MoravoSeed is now involved in the establishment of a molecular laboratory through an interregional cooperation, funded by the EC, to enhance the competitiveness of Czech and Slovak vegetable producers.

Ongoing activities focus on breeding by negative or positive selection with 100 working genotypes, mainly of local origin. Challenges for breeding garlic include its instability due to climate changes, whereby some traits can be lost in time (i.e. number of cloves is decreasing, since winters are shorter and milder). The plasticity of the varieties is very important, in terms of climate, latitude and altitude. It is important to find resistance against biotic and abiotic factors, especially drought. Length of vegetation is also important, with long vegetation required. Challenges for breeding also come from changed legislation, since the number of allowed phytochemicals is continuously reduced, often without suitable alternatives. Also virus-free bulbs are required for registration, and this is sometimes rather impractical and would require more tolerance.

MoravoSeed cooperates with the Czech genebank, but not with foreign genebanks. Strengthened cooperation would call for simplification of the legislative/administrative process involved in requesting genebank accessions. Information-rich online catalogues are also appreciated, ideally with genotypic descriptions and the main traits, such as resistances and tolerances. It should be possible to work together in the future to use CRISPR aiming to switch garlic to flowering and thus make crosses and build the ideal garlic. Breeders are usually very open to cooperation since they constantly need new material.

The ideal garlic today is a variety that is resistant to drought, tolerant to virus diseases, has a long vegetation period, 8–10 cloves, is non-attractive for nematodes and has high plasticity and long shelf life.

Discussion on proposals for ECPGR activities, cooperation with other Working Groups, other

(Introduced by H. Stavělíková)

European Allium Database

It was agreed that it should be maintained on the website, with a note indicating that it was no longer updated since 2012 and pointing at EURISCO for updated data. Countries are encouraged to transfer all their data to EURISCO.

Safety duplication

Olomouc, Czech Republic, can host accessions for safety duplication. No other institute offered space, but Working Group members are invited to keep the group informed in case of availability. Safety duplication can be part of the strategy that the *Allium* Working Group intends to develop through the Grant Scheme proposal.

Cryopreservation

It will not be necessary that each institution gets involved in cryopreservation. A few institutions with larger expertise could take the lead and host material for the others. A

strategy on how to organize cryopreservation for *Allium* across the region could be one of the results of the project to be submitted under the Grant Scheme¹. Also other Working Groups dealing with vegetatively propagated crops may be involved in discussing such strategy. The establishment of a core collection could be the way to go to prioritize the material that needs to be cryopreserved.

Project proposal to be submitted under the ECPGR Grant Scheme

M. Nagel offered to coordinate the proposal to submit for funding an activity that could involve both the *Allium* and the Cryopreservation WGs, using garlic as a pilot crop to develop a vision of what could be done in terms of political strategy, technology needs and definition of material to be cryopreserved. A conservation strategy for garlic (and shallot) can be based on a systematic genotyping of collections made available by the partners. Ideally, such initiative could be upscaled through a Horizon Europe project, should the opportunity arise.

Taxonomy collection at IPK

The WG believes that this is a very precious collection, that should be maintained. Interest for the breeders could be raised if seed were made available. It would be necessary to isolate the flowers to avoid interbreeding among species and sterility of the seeds.

Conclusion

Staff of the National Institute for Horticultural Research (InHort) were praised and thanked for the excellent organization of the meeting and the great hospitality.

¹ The Activity proposal 'Garli-CCS' (Genotyping-by-sequencing of the European garlic collection to develop a sustainable *ex situ* conservation strategy), was submitted under the Sixth Call for proposal of the ECPGR Grant Scheme and awarded for funding. The Activity aims to develop an efficient conservation strategy for European garlic accessions in conjunction with cryopreservation and increased access for breeders and producers through prioritization, rationalization and acquisition activities.

ANNEXES

Annex 1 – Agenda

Eighth Meeting of the Allium Working Group

11-12 October 2022, Skierniewice, Poland

Agenda

Monday 10 October

Arrival of meeting participants INHORT will provide transport for participants from the Chopin Airport, Modlin Airport and the train station.

Tuesday 11 October

9:00-9:10	Welcome by the WG Chair and short introduction of all participants (H.
	Stavelikova)
9:10-9:20	Welcome address by the Director of the National Institute for Horticultural
	Research (D. Konopacka)
9:20-9:30	Information from local organizer (M. Chojnowski, M. Sitarek)
9:30-9:45	Information on the ECPGR Programme (L. Maggioni)
9:45-10:00	Report on the work of the Working Group over the past years – (<i>H. Stavelikova</i>)
10:00 - 10:10	Results of SafeAlliDiv project – (L. Lepse)
10:10 - 10:20	Discussion
10:20-10:45	Coffee break
10:45- 12:00	Report on national Allium collections from each participant (ca. 10 min each)
	Czech Republic (<i>H. Stavělíková</i>), Estonia (<i>P. Põldma</i>), Finland (<i>T. Suojala-Ahlfors</i>)
	Germany (M. Nagel), Greece (C. Margaret Cook), Italy (A. Natalini), Latvia (Liga
	Lepse)
12:00-13:00	Lunch
13:00-14:00	The Netherlands (R. Lievers/C. Kik), Norway (I. M. Vågen), Poland (M.
	Chojnowski), Portugal (I. Gomes da Silva), Slovenia (J. Šuštar Vozlič), Spain (M. de
	los Mozos Pascual)
14:00-14:25	Discussion
14:25-14:55	Coffee break
14:55-15:20	Cryopreservation - state of the art for vegetatively propagated Alliums (Co-
	Chair of the Cryopreservation WG – M. Faltus)
15:20-16:00	Discussion
16:00-17:30	Visiting the Regional Centre for Horticultural Biodiversity
19:30	Social dinner – Dworek hotel

Wednesday 12 October 2022

9:00-9:20	Overview of the global Allium collections (C. Kik)
9:20-9:50	The European Allum Database (status and perspectives) (Discussion introduced
	by M. Nagel)
9:50-10:10	The breeder's perspective (main needs and expectations from genetic
	resources) (L. Nedorost, MoravoSeed, Czech Republic)
10:10-10:30	Discussion
10:30-10:50	Coffee Break
10:50-11:45	Discussion on proposals for ECPGR activities, cooperation with other working
	groups, other (introduced by H. Stavělíková)
11:45-12:00	Wrap-up and conclusion (H. Stavělíková)
12:00-13:00	Lunch
13:00-17:00	Visiting the collections of genetic resources of fruit trees and the Museum in
	Nieborów (http://www.nieborow.art.pl/en/), where the reconstruction of
	palace kitchen gardens has been made, using genetic resources collected by the
	National Institute for Horticultural Research

Thursday 13 October 2022

Departure of participants

INHORT will provide transport for participants to the Chopin Airport, Modlin Airport and the train station.

Annex 2 – List of participants

Eighth Meeting of the Allium Working Group

11-12 October 2022, Skierniewice, Poland

List of participants

Working group members

Helena Stavělíková **Chair** Crop Research Institute, Genetic Resources for Vegetables, Medicinal and Special Plants Šlechtitelů 29 783 71 Olomouc **Czech Republic** Tel (420) 585 208 965 Email: <u>stavelikova@genobanka.cz</u>

Priit Põldma Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences Kreutzwaldi 5 51006 Tartu **Estonia** Tel (372) 7313517 Email: <u>priit.poldma@emu.ee</u>

Terhi Suojala-Ahlfors Natural Resources Institute Finland (Luke) Toivonlinnantie 518 FI-21500 Piikkiö **Finland** Tel (358) 29 5326557 Email: <u>terhi.suojala-ahlfors@luke.fi</u>

Manuela Nagel Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) OT Gatersleben 06466 Stadt Seeland **Germany** Tel (49) (0)39482 5156 Email: <u>nagel@ipk-gatersleben.de</u> Catherine Margaret Cook Institute of Plant Breeding and Genetic Resources Hellenic Agricultural Organization - DIMITRA PO Box 60458; 57001 Thermi, Thessaloniki **Greece** Tel (30) 2310 471110 Emails: <u>ccook@elgo.gr; cook@bio.auth.gr</u>

Alessandro Natalini Council for Agricultural Research and Economics -Research Centre for Vegetables and Ornamental Crops (CREA-OF) Strada Statale 4 Via Salaria, 1 63030 Monsampolo del Tronto **Italy** Tel (39) 0735 701706 Email: <u>alessandro.natalini@crea.gov.it</u>

Līga Lepse Institute of Horticulture Grandu iela 1, Ceriņi, Krimūnu pagasts 3701 Dobeles novads Latvia Tel (371) 26185596 Email: <u>liga.lepse@llu.lv</u>

Chris Kik Centre for Genetic Resources, the Netherlands (CGN) 6700 AA Wageningen **The Netherlands** Tel (31-317) 480861 Email: <u>chris.kik@wur.nl</u> Rik Lievers Centre for Genetic Resources, the Netherlands (CGN) 6700 AA Wageningen **Netherlands** Tel (31) 317487748 Email: <u>rik.lievers@wur.nl</u>

Ingunn M. Vågen Norwegian Institute of Bioeconomy Research (NIBIO) 1431 Ås **Norway** Tel (47) 40622904 Email: <u>ingunn.vaagen@nibio.no</u>

Isabel Gomes da Silva Intituto Nacional de Investigação Agrária e Veterinária (INIAV), Banco Português de Germoplasma Vegetal Quinta de S. José. S. Pedro de Merelim 4700-859 Braga **Portugal** Tel (351) 253198730 Email: <u>isabel.silva@iniav.pt</u>

Jelka Šuštar Vozlič Agricultural Institute of Slovenia Hacquetova ulica 17 1000 Ljubljana **Slovenia** Tel (386) 1 2805188 Email: <u>jelka.sustar-vozlic@kis.si</u>

Marcelino de los Mozos Pascual Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla – La Mancha (IRIAF). Centro de Investigación Agroforestal de Albaladejito Ctra. Toledo-Cuenca, km. 174 16194 Cuenca **Spain** Tel (34) 969177767 Email: <u>mde@jccm.es</u>

Observers

Eudovít Nedorost Moravoseed CZ a.s. Mušlov 1701/4 Mikulov, 692 01 **Czech Republic** Tel (420) 602697949 Email: <u>nedorost@moravoseed.cz</u>

Miloš Faltus Co-Chair of Cryopreservation WG Výzkumný ústav rostlinné výroby, v.v.i. Praha (VÚRV) Crop Research Institute 161 06 Prague, Ruzyne 507 **Czech Republic** Tel (42) 2 33022362 Email: <u>faltus@vurv.cz</u>

Observers from the National Institute for Horticultural Research Konstytucji 3 Maja 1/3 96-100 Skierniewice **Poland** Anna Bakalarska Tel (48) 516124653 Email: <u>anna.bakalarska@inhort.pl</u>

Maria Burian Tel (48) 606451397 Email: <u>maria.burian@inhort.pl</u>

Mariusz Chojnowski Tel (48) 606268864 Email: <u>mariusz.chojnowski@inhort.pl</u>

Denise Fu Dostatny Tel (48) 504845182 Email:<u>denise.dostatny@inhort.pl</u>

Waldemar Kiszczak Tel (48) 468333279 Email: <u>waldemar.kiszczak@inhort.pl</u>

Miroslaw Sitarek Tel (48) 46834 439 Email: <u>miroslaw.sitarek@inhort.pl</u>

Anna Wawrzyniak Tel (48) 468346520 <u>Email: anna.wawrzyniak@inhort.pl</u>

ECPGR Secretariat

Lorenzo Maggioni Alliance Bioversity International and CIAT Via di San Domenico, 1 00153 Rome Italy Tel: (39) 066118231 Email: <u>l.maggioni@cgiar.org</u>

Remote participants

Six remote participants connected during the meeting.