ECPGR Activity Grant Scheme
Proposal Form

Sixth Call – Phase X (2019-2023)

Activity Proposal

Activity	
Full title	Genotyping-by-sequencing of the European garlic collection to develop a sustainable *ex situ* conservation strategy
Acronym (or short title)	Garli-CCS (Garlic Cryopreservation & Conservation Strategy)
Duration of Activity (in months)	24 months
Start date – End date *Please indicate start date not earlier than 3 months after deadline of Call*	1st April 2023

Applying Working Group(s)

	Working Group	Indicate name and surname of Working Group Chair
1.	Allium	Helena Stavělíková
2.	Cryopreservation	Bart Panis and Miloš Faltus
3	Documentation and Information	Stephan Weise

Activity Coordinator

Activity Coordinator	
Name and Surname	Manuela Nagel
Working Group	Allium Working Group
Nationality	German
Current position	Head of Cryo and Stress Biology Research Group
Institute	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
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Activity Partners (ECPGR-funded)

Please note that each partner needs to be a member of an ECPGR Working Group to be eligible for funding. For self-funded partners please use the separate box below.

Partner ID No.	Name and Surname	Institute	Country
1	Manuela Nagel	IPK	Germany
2	Līga Lepse	Institute of Horticulture	Latvia
3	Helena Stavělíková	Crop Research Institute	Czech Republic
4	Terhi Suojala-Ahlfors	Natural Resources Institute Finland (Luke)	Finland
5	Priit Põldma	Estonian University of Life Sciences	Estonia
6	Florence Esnault	INRAE	France
7	Danguolė Juškevičienė	Lithuanian Research Centre for Agriculture and Forestry (LAMMC)	Lithuania
8	Jelka Šuštar Vozlič, Mojca Škof	Agricultural Institute of Slovenia	Slovenia
9	Catherine Cook	Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization-Dimitra	Greece
10	Smiljana Goreta Ban	Institute of Agriculture and Tourism	Croatia
11	Isabel Gomes da Silva	Portuguese Genebank (BPGV)	Portugal
12	Marcelino de_los_Mozos Pascual	Regional Institute for Research and Agrifood and Forestry Development of Castilla - La Mancha (IRIAF)	Spain
13	Vincenzo Candido	Department of European and Mediterranean Cultures - DiCEM	Italia
14	Miloš Faltus	Crop Research Institute	Czech Republic
15	Mariusz Chojnowski, Denise Fu Dostatny, Anna Wawrzyniak	National Institute for Horticultural Research (INHORT)	Poland

16	Erik de Vahl	Swedish National Genebank for Vegetatively Propagated Horticultural Crops	Sweden

Self-funded partners

Partner No.	Name and Surname	Institute	Country
1	Stephan Weise	IPK / EURISCO	Germany
2	Domingo José Rios Mesa, Desirée Afonso Morales	Center for Conservation of Agrobiodiversity of Tenerife (CCBAT)	Spain
3	María Victoria Colombo Rodríguez and Isabel Fernández Navarro	Madrid Institute for Rural, Agricultural and Food Research and Development (IMIDRA)	Spain
4	María Cristina Alcántara Rodríguez	Regional Institute for Research and Agrifood and Forestry Development of Castilla - La Mancha (IRIAF)	Spain
5	~~Mariusz Chojnowski, Denise Fu Dostatny, Anna Wawrzyniak~~ Maarten van Zonneveld	~~National Institute for Horticultural Research (INHORT)~~ World Vegetable Center	~~Poland~~ International

Description of Activity (suggested max. 1000 words)

Please address the following aspects:

– ***Background:*** Explain the context behind the choice of this Activity, e.g. **why this has been prioritized or selected**. If this is the continuation of a preceding Activity, please indicate how and why the new Activity will build on previous results/experiences.

Garlic is an important crop in Europe. About 870 thousand tons are produced annually, in particular in Spain (269 thousand tons), Ukraine (211 thousand tons) and Russia (189 thousand tons). However, most bulbs are imported from China (having a tons production volume of 22.3 million tons) and Egypt (https://www.fao.org/faostat/). As demand for high-quality garlic bulbs increases, and import and transport costs rise, **garlic breeding and production in Europe becomes more interesting** (www.feshplaza.de). Therefore, gene banks need to support these activities by providing valuable genetic resources for breeding and/or production.

About 3,200 vegetatively propagated accessions of garlic are preserved in European genebanks. The largest collections are found in Spain (752 accessions), the Czech Republic (631 accessions) and Poland (531 accessions) (https://eurisco.ipk-gatersleben.de/). Most of the **garlic accessions** are maintained in the field collections and **require annual replantation. Therefore, the maintenance in the field is very time-consuming and costly (Keller and Kik, 2018)**. Although in the field genebanks plants can adapt to the local and changing environmental conditions, they are also exposed to stresses such as extreme weather conditions and infestation by insects and pathogens. Therefore, **the safety duplication** in different field sites, in *in vitro* slow-growth storage or by **cryopreservation** (FAO, 2014; Panis et al., 2020), **is an essential strategy to conserve the valuable genebank material** in the long-term.

Cryopreservation is the only approach for long-term conservation of clonal plant genetic resources. In the case of garlic, often shoot tips are excised from bulbils, cloves or *in vitro* plantlets and subjected to a so-called vitrification procedure that predominantly uses Plant Vitrification Solution 3 (PVS3) as a cryoprotectant (Keller and Senula, 2012; Kim et al., 2012). When pretreated tissues are exposed to liquid nitrogen, the cell cytoplasms vitrify (Wang et al., 2020) and the shoot tips can be safely stored under ultra-low temperature (<150 °C); and are able to regrow to a fully developed plant after thawing. More than 1,700 garlic accessions have been already cryopreserved worldwide. According to Acker et al. (2017), the largest collections in cryo are in South Korea (1,158 accessions), Germany (200 garlic and 45 shallot accessions), (Poland (188 accessions) and

the Czech Republic (127 accessions). Between 2007 and 2011, as part of the EURALLIVEG project (EC 870/2004, Blattner et al. (2012)), the collaborative initiative for the cryopreservation of garlic in Europe was initiated and **a first model for safety duplication using cryopreservation was introduced**. As a result, 202 European Allium accessions provided by national collections from Germany, the Czech Republic, Poland, Italy, France and Scandinavia were safely cryopreserved and duplicated off-site at IPK (Germany), InHORT (Poland) and CRI (Czech Republic). However, at this stage, accessions were selected based on passport data and morphological characteristics. **In order to safely duplicate further unique garlic accessions, genotyping must first be carried out**, followed by prioritization of important garlic accessions and, in the third step, cryopreservation must be organized.

High-throughput identification of molecular markers, i.e. Single Nucleotide Polymorphism (SNP), by using Genotyping-By-Sequencing (**GBS) has become an important tool for genetic analysis**. In addition, **a chromosome-level genome assembly was published for garlic in 2020**. This revealed that garlic has a large diploid genome (2n = 2x = 16) of total size of approximately 16.24 Gbp and the annotation of 57,561 predicted protein-coding genes (Sun et al., 2020). Combining the available reference genome and GBS technology, the data obtained will provide a valuable new resource for research in garlic biology and breeding. So far, by using GBS technology, the first garlic germplasm collection (417 accessions) was genotyped in 2017; and 286 unique and 131 redundant accessions were identified (Egea et al., 2017). **However, no prioritization, safety duplication, cryopreservation and utilization strategy has been implemented yet.**

The aim of the activity is to develop an efficient conservation strategy for European garlic accessions in conjunction with the implementation of cryopreservation and increased usability of garlic accessions for breeders and producers through prioritization, rationalization and acquisition activities.

To develop a garlic conservation strategy, a number of considerations need to be taken into account:

- Duplicates of garlic accessions are present in different genebanks due to intensive exchange in the past.
- Identification of unique or duplicate accessions is difficult by morphological descriptors.
- Cryopreservation takes time and training is necessary; prioritization of valuable genotypes to be conserved first is required.
- Better usability will be achieved by the development of a well-characterized collection.

Consequently, a number of aspects need to be addressed:

- First, criteria need to be established for unique accessions, i.e. number of unique alleles to be prioritized for cryopreservation and considered for a garlic core collection.
- Second, criteria need to be established for duplicated accessions, i.e. number of identical alleles, that can serve as a basis for rationalization or further cryopreservation.
- Third, cryopreservation training needs to be carried out to enable genebanks to cryopreserve their collections.
- Fourth, gap analysis should be considered and an acquisition strategy suggested.
- Fifth, a comprehensive conservation strategy involving cryopreservation need to be developed.

The planned project builds up on

1. Intensive and successful collaborations between members of the *Allium* working group of ECPGR who conducted e.g. genotyping of potato onion within the SafeAlliDiv project using ~12 SSR markers (Ruņģis et al., 2020)
    - The planned project will make use of the fruitful collaboration and
    - generate between 1,500 and 7,200 SNP markers using genotyping-by-sequencing (GBS)

2. The EURALLIVEG project. Based on passport, morphological and image data, 202 accessions were selected for cryopreservation.
    - The planned project will prioritize European accessions for cryopreservation based on genetic markers
    - The ECPGR Cryopreservation group will support activities by providing cryopreservation training and developing a cryopreservation strategy.

3. Experiences available by partners (F. Blattner, D. Harpke) at IPK who fully sequenced the garlic and shallot collections and showed that garlic subgroups clustered according to the infraspecific classification published by Maaß and Klaas (1995). IPK partners are willing to fully support the project by technical assistance for DNA extraction (F. Blattner), phenotyping (M. Nagel) and data accessibility (S. Weise).

– ***Justification:*** Explain why this Activity is justified in terms of making progress towards achieving the ECPGR objectives.

1. To **efficiently conserve and provide access to unique germplasm in Europe** through AEGIS and the European Collection
    - After genotyping, data analysis, setting criteria, unique garlic accessions can be determined, made accessible through AEGIS and prioritized for cryopreservation and a core collection.
    - Cryopreservation training will be provided to enable rapid safety duplication in cryo.
    - Genotypic and phenotypic information will be available via EURISCO and platforms storing genomic data, i.e. EMBL-EVA

2. To provide passport and **phenotypic information** of actively conserved European PGRFA diversity ex situ and in situ through the EURISCO catalogue
    - Besides the link for the genotypic data, additional phenotypic data (1 or 2 characters) will be recorded and provided for inclusion to the respective National Focal Points for EURISCO.

3. To promote use of garlic GRFA
    - By providing genotypic data and phenotypic data relevant for production and breeding, we will increase the effectiveness of selection of suitable genotypes.
    - Marker-trait association generated by genome-wide association (GWAS) analysis will stimulate marker-assisted selection and innovations in breeding procedures.

– ***Rationale for the choice of partners:*** Explain why the selected partners are the most suitable to carry out the proposed Activity and briefly describe their respective roles in the Activity.
Partners: All nominated partners have extensive knowledge in the maintenance of garlic genetic resources and will contribute to the development of the garlic conservation strategy.

Partner 1. Manuela Nagel (curator, seed and cryopreservation specialist), **F. Blattner and D. Harpke** (taxonomists) and **Stephan Weise** (EURISCO; documentation specialist) are research group leaders at IPK genebank and heads of the Cryo and Stress Biology group (M. Nagel), Experimental Taxonomy group (F. Blattner) and Documentation group (S. Weise). All have long-term experience in maintenance, evaluation and documentation of plant genetic resources and genotyped the IPK garlic and shallot collection in a collaborative activity. In the frame of the Garli-CCS project, they will conduct the DNA extraction, genotyping (library preparation and sequencing (1x120bp) per sample) of the European garlic accessions, data analysis (GBS and GWAS), phenotyping of IPK collection and support data publication and accessibility. Further, they will contribute to the discussion on the cryopreservation and conservation strategy.

Partner 2. Liga Lepse (curator), manager of the garlic and shallot collection in Latvia, has experience of more than 20 years of maintenance and evaluation of collections. She has experience in management and leading of international collaboration projects, including ECPGR grants.

Partner 3. **Helena Stavělíková** (curator) works at the Crop Research Institute. She has worked with *Allium* germplasm for 30 years. She has been a curator of the *Allium* collections for 21 years. In her work, she deals with the registration, description and cultivation of genetic resources of garlic. Since 2015, she is the chairperson of the ECPGR Allium working group and participates in national and international projects.

Partner 4. Terhi Suojala-Ahlfors (curator/crop specialist) works at the Natural Resources Institute Finland (Luke). She has worked with field collections of *Allium* species for 20 years and has good connections to practical farming in Finland. She has experience on Nordic co-operation in PGR work (NordGen working groups) and she participated in the previous ECPGR-granted *Allium* activity (SafeAlliDiv).

Partner 5. Priit Põldma (crop specialist) is researcher at Estonian University of Life Sciences. He has over 20 years of experience in garlic/onion experimental work. As a horticultural adviser, he has good connections to Estonian farmers and has a small collection of local garlic clones.

Partner 6. Florence Esnault, manager of the garlic and shallot collection in France, has experience in managing vegetative Allium and potato genetic resources collections, in analyzing genetic diversity using

molecular data and participated in the EURALLIVEG project. She will provide leaves of the French garlic accessions for genotyping, phenotypic data of this collection and contribute to the discussion on the conservation strategy.

Partner 7. Danguolė Juškevičienė (curator, breeder) is researcher at the Lithuanian Research Centre for Agriculture and Forestry. More than 20 years, she has been working with the genetic resources of Allium plants and developing new cultivars. She is co-author of 3 Lithuanin garlic cultivars and 1 onion cultivar.

Partner 8. Jelka Šuštar Vozlič (curator, gene bank manager), researcher of genetics and plant breeding at the Agricultural Institute of Slovenia (AIS), including application of molecular markers to diversity studies of vegetable genetic resources. **Mojca Škof** (curator, crop specialist) manager of the garlic and onion collections in Slovenia, with more than 30 years of experience in evaluation of horticultural crop species, including *Allium*. AIS will provide leaves of the Slovenian garlic accessions for genotyping, phenotypic data of the collection and contribute to the discussion on the conservation strategy.

Partner 9. Catherine Cook (curator) is a researcher at the Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organisation-Dimitra, Greece with ten years on the Managing committee of the Greek Genebank. She works on the chemical diversity of plant genetic resources and is representative for the *Allium* collection. She will provide leaf material for sequencing and phenotypic data of Greek garlic accessions (in kind contribution) and take part in development of conservation strategy

Partner 10. Smiljana Goreta Ban (curator/crop specialist) works at the Institute of Agriculture and Tourism in Croatia. She has long experience in managing vegetative garlic and shallot field collections and their phenotypic and biochemical characterization. She has participated in the previous ECPGR-granted *Allium* activity (SafeAlliDiv) and coordinates ECPGR-granted *Brassica* activity (EUBRASSWILD).

Partner 11. Isabel Gomes da Silva (curator) works in the Portuguese Genebank. She has been responsible for Allium collections and has experience in maintenance, characterization and evaluation of genetic resources of garlic and has participated in national and international projects.

Partner 12. **Marcelino de_los_Mozos Pascual** has been curator of the Bank of Plant Germplasm of the Agroforestry Research Center of Albaladejito (IRIAF-CIAF) in Cuenca, Spain during the last 25 years. He is working on *ex situ* conservation and morpho-agronomic characterization and evaluation of different crops (mainly grain legumes, saffron and other crocuses and also different *Allium* species, including the most important Spanish garlic collection). He participated and/or coordinated several national and international projects about conservation, management and characterization/evaluation of genetic resources. In the development of the different proposed tasks, **María Cristina Alcántara Ramírez** will also participate. She is crop specialist with a wide experience in herbaceous crops, including garlic, and currently involved in the field management of collections of the genebank. Partner 12 will be involved in the provision of leaf material for sequencing and also passport and phenotypic data from the IRIAF garlic collection. This work will be tightly coordinated with other Spanish genebanks preserving garlic materials (self-funded partners 2 and 3) to avoid duplicities and economize resources. Will also participate in the development of garlic preservation strategy and cryopreservation training.

Partner 13. **Vincenzo Candido**, professor of Vegetable Crops and Floriculture at the Department of European and Mediterranean Cultures (DiCEM) of Basilicata University (Italy). Among his research topics he has been involved in the agronomic evaluation of germplasm collections of several vegetable species. He is a member of the Italian Academy of Mediterranean Biodiversity Sciences (ASBM) and he has participated at the Scientific and Organizing Committees of several Italian Congresses on Biodiversity. During the last 20 years, he has curated the Italian garlic collection; besides, he has collaborated at EURALLIVEG "Vegetative Allium, Europe's Core Collection, safe & sound" Project.

Partner 14. **Miloš Faltus** (cryopreservation specialist) works at the Crop Research Institute (CRI). He has developed and used cryopreservation methods on plant genetic resources of vegetatively propagated crops for 20 years. He is a head of the Czech Crop Cryobank in Prague (CRI). In his work, he deals with the tissue cultures of plant genetic resources of vegetatively propagated crops. He is an expert in thermal analysis use for plant cryopreservation methods development and utilization. Since 2022, he is the co-chair of the ECPGR working group for cryopreservation and participates in national and international projects. He will arrange an

advanced practical course on garlic germplasm cryopreservation for partners dealing with garlic germplasm cryopreservation and/or tissue culture collections.

Self-funded Partner 2. CCBAT is the only Spanish genebank preserving garlic accessions adapted to subtropical conditions. The working team of the CCBAT is composed by **Domingo José Rios Mesa** (manager of the bank) and **Desirée Afonso Morales** (crop specialist responsible for the maintenance of several germplasm collections in CCBAT). Both specialists have wide experience in the collection, preservation and characterization of genetic resources, including garlic, and also participation and/or coordination of research projects on this crop. This partner will provide leaf material for sequencing and also passport and phenotypic data from garlic materials collected in the Canary Islands. Both specialists will also participate in the development of garlic preservation strategy and if possible in cryopreservation training.

Self-funded Partner 3. IMIDRA preserves important germplasm collections of grapevines and forest species, and also an interesting collection of traditional horticultural plants from the Authonomous Region of Madrid, including garlic. The team for this proposal is composed by **María Victoria Colombo Rodríguez** (manager of the IMIDRA horticultural genebank) and **Isabel Fernández Navarro** (specialist in horticultural crops), with wide experience in preservation and characterization of garlic. This partner will provide leaf material for sequencing and also passport and phenotypic data from local cultivars of garlic collected in the Autonomous Region of Madrid. Both specialists will also participate in the development of garlic preservation strategy and if possible in cryopreservation training.

Self-funded Partner 5. INHORT preserves germplasm collections of horticultural crops, including over 600 garlic accessions from 36 countries, half of them are Polish landraces. Mariusz Chojnowski is the vegetable crop curator in INHORT Skierniewice, Denise Fu Dostatny is the curator for WCR and Anna Wawrzyniak for the Allium field collections and Maria Burian is cryopreservation specialist. They are ready to provide material for genotyping, verified passport data as well as conduct phenotyping about 300 Polish landraces and would like to participate in all online meetings but also in physical meetings as a self-funded participants.

– Methodology or Approach: Explain how the partners will operate. Clearly explain who is expected to do what. Also explain the rationale of meeting (or not) as part of the Activity. Include a Gantt Chart, to illustrate the work breakdown structure of the project.

To develop a conservation strategy for garlic genetic resources, we aim to genotype the European garlic collections and to identify unique and duplicated accessions which will be prioritized for cryopreservation and for phenotyping in the frame of a core collection. To genotype the European garlic approach, we will select 1,800 accessions (~ 30,000 EUR) from the National Inventories. We aim to use unique, (non-duplicated) material that is either from the center of origin or a landrace of the provider country.

To develop the strategy, the work is conducted with five work packages:

1. **Sequencing (April – December 2023).**
   WP 1.1 Partners (2 – 13) will collect leaf material from young single plants in April – June 2023, dry over silica gel and post to Partner 1.
   WP 1.2 Partner 1 (M. Nagel, F. Blattner and D. Harpke) will extract DNA, submit DNA to sequencing (F. Blattner and D. Harpke). After the marker information will be generated, the data will be stored in existing database structures of the IPK and can be made immediately available to material providers on request.
   WP 1.3 Partner 1 (F. Blattner and D. Harpke) will analyze marker data and provide results for discussions.

2. **Phenotyping (April – September 2023 and 2024)**
   WP 2.1. To increase the attractiveness of the accessions and the breeding value of the material, garlic accessions used for sequencing will be phenotyped by **Partners 1- 13** for the traits; a) Ability to produce scape (7.2.1), b) Bulb structure type (7.1.20) and c) Shape of the mature bulb (7.1.12). The phenotyping data will be compiled and the respective National Inventory Focal Points will be approached to submit the data to EURISCO.
   WP 2.2. The combination of phenotyping and sequencing data will also enable a genome-wide association (GWAS) analysis, conducted by **Partner 1** (M. Nagel), and identify marker-trait

association and loci responsible for bulb weight and size. This information will stimulate further breeding processes and support activities such as marker-assisted selection. The results of the GWAS will be made available via an open-access publication.

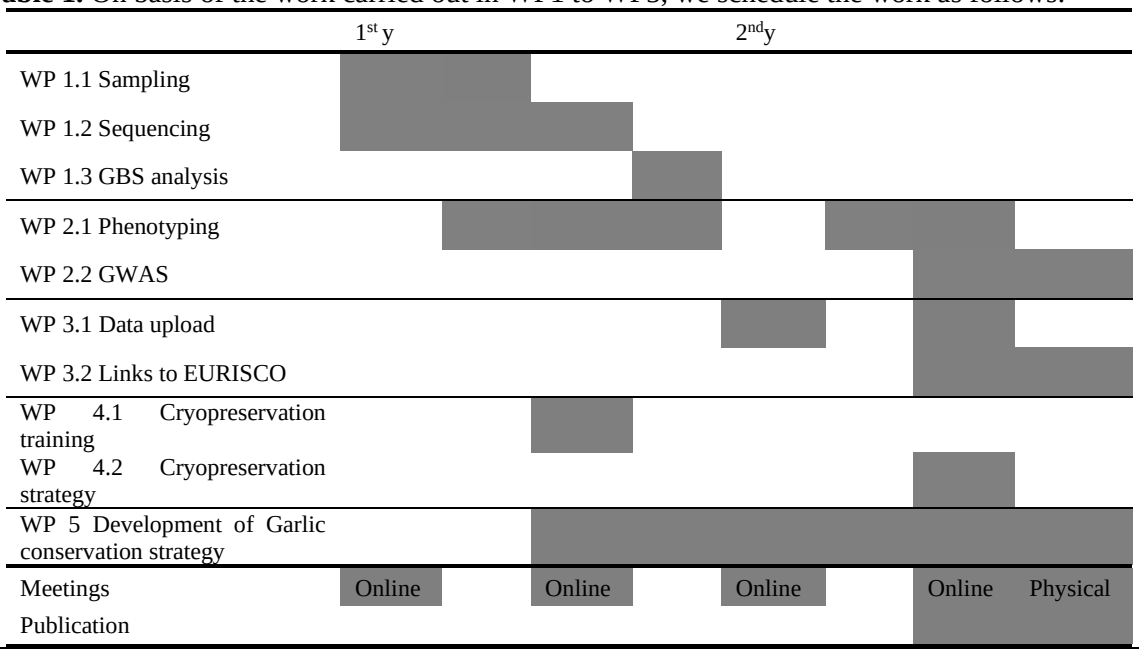
3. **Data accessibility (December 2023 and 2024)**
   WP 3.1 After evaluation and verification, **Partner 1** (S. Weise) will upload the data to the EMBL-EVA public repository.
   WP 3.2 Subsequently, links will be established in EURISCO between the corresponding accessions documented there and the genotyping data.

4. **Cryopreservation training (October 2023 - December 2024)**
   WP 4.1 Partner 14 representing the CRYOPRESERVATION group will organize for the consortium members, a cryopreservation workshop in Prague. Overall, 7 participants and 2 experts from abroad will be invited to discuss procedures, provide guidance and training on garlic cryopreservation. Other partners can join the lectures online.

   WP 4.2. All partners implementing cryopreservation will be involved to develop a **cryopreservation strategy** which includes thresholds for the number of shoot tips for safety duplicates, the number of replicates, locations for safety duplications and additional support by online training and meetings. The cryopreservation strategy should be considered a model for clonal plants. Therefore, a two-day virtual meeting is planned. The first day of the meeting will summarize the experiences of the workshop organizers and participants. The second day will be devoted to a discussion of the cryopreservation strategy. The number of participants will not be limited. This meeting will take place at the end of 2024.

5. **Garlic conservation strategy (Whole project period)**
   WP 5. All partners will discuss a conservation strategy in **four online meetings** taking place in the 1., 6-7th., 12-14th and 18-20th project months and **one final face-to-face meeting at IPK in 22-24th months**. GBS data available from IPK collection will be used as the preliminary dataset for discussion of the following topics
   - Determinants and thresholds for unique accessions
   - Determinants and thresholds for duplicate accessions
   - Prioritization of garlic accessions for cryopreservation
   - Prioritization of garlic accessions for a European Core Collection
   - Gap analysis and acquisition strategy

Table 1. On basis of the work carried out in WP1 to WP5, we schedule the work as follows:



– ***Description of genetic material:*** If your Activity is focusing on genetic material, please describe in detail, as far as possible, who is providing this genetic material, its status and the number of accessions under investigation (for example: *This Activity aims at molecularly analyzing / safety-duplicating / evaluating / collecting XY accessions (listed) of "Genus species", provided by genebank Z/ farmers in country W /to be collected in country P…, etc.*).

In the first online meeting, the partners will agree on the garlic accessions delivered to IPK for genotyping. In total, 1,800 accessions can be analyzed.

- Partner 1 (IPK, Germany) will provide sequence information and phenotypic data of 460 garlic accessions.
- Partner 2 (LatHort, Latvia) will provide leaf material for sequencing and phenotypic data of Latvian genotypes (max. 92 garlic accessions).
- Partner 3 (CRI, Czech Republic) will provide leaf material for sequencing and phenotypic data (max. 605 garlic accessions).
- Partner 4 (Luke, Finland) will provide leaf material for sequencing and phenotypic data of Finnish material (max. 7 garlic accessions).
- Partner 5 (EMU, Estonia) will provide leaf material for sequencing and phenotypic data of Estonian material (max. 13 garlic accessions).
- Partner 6 (INRAE, France) will provide leaf material for genotyping and phenotypic data of 110 garlic accessions.
- Partner 7 (LAMMC, Lithuania) will provide leaf material for sequencing and phenotyping data of Lithuanian garlic material (max. 56 garlic accessions)
- Partner 8 (AIS, Slovenia) will provide leaf material for sequencing and phenotypic data of Slovenian garlic accessions (max. 50 garlic accessions).
- Partner 9 (IPBGR, Greece) will provide leaf material for sequencing and phenotypic data of Greek garlic accessions (in kind contribution, max. 20 accessions).
- Partner 10 (IPTPO, Croatia) will provide leaf material for sequencing and phenotypic data of Croatian collection (max. 118 garlic accessions).
- Partner 11 (INIAV, Portugal) will provide leaf material for sequencing and phenotypic data of Portugal garlic accessions (max. 60 accessions) and take part in development of conservation strategy and cryopreservation training.
- Partner 12 (IRIAF-CIAF, Spain) will provide leaf material for sequencing and also passport and phenotypic data from a selection of approximately 300 accessions of different origins preserved in the garlic collection of IRIAF-CIAF Albaladejito.

- Partner 13 (DiCEM, Italy) will provide leaf material for sequencing of 50 garlic accessions belonging to the Italian collection.

- Self-funded partner 2 (Spain) will provide leaf material for sequencing and also passport and phenotypic data from a selected collection of 35-40 accessions of garlic collected in the Canary Islands. These tasks will be made in close collaboration with partner 12 to avoid duplicities.

- Self-funded partner 3 (Spain) will provide leaf material for sequencing and also passport and phenotypic data from 15-20 accessions of traditional varieties of garlic growing in the province of Madrid. These tasks will be made in close collaboration with partner 12 to avoid duplicities. Participation in the development of garlic preservation strategy and cryopreservation training.

- Self-funded partner 5 (INHORT, Poland) can provide leaf material for sequencing and also passport and phenotypic data from 300 garlic accessions

– *Expected impact.* Clearly specify the expected impact from this Activity for the respective ECPGR objective(s), compared to the current state of progress of those same objectives. Explain how the impact will be obtained.

The garlic conservation strategy will support decision-making processes in genebanks in order

- to prioritize garlic accession for cryopreservation and/or other safety duplication approaches
- to develop core collections for preferential use in future
- to find duplicates and rationalize collections
- to support cryopreservation and safety duplication activities

It will further increase the usability of the material by providing

- sequencing data to breeders and researchers
- to conduct GWAS on important agronomic traits
- to complement the phenotypic data available in EURISCO

– *Links with other non-ECPGR projects or individuals*: If applicable, clearly explain the objectives of the linked projects and the reasons for complementarity with the ECPGR Activity.

Not applicable

Expected products and related ECPGR Objectives

List concrete products and results that are obtained by the Activity and the corresponding number(s) of the ECPGR Outcome(s) and/or Output(s) and/or Activities to which each product/result will contribute.

	Expected products/results	Corresponding ECPGR output, activity
1	Publicly available sequencing data of 1,800 garlic accessions	To promote use of PGRFA
2	Additional phenotypic data for ability to produce scape, bulb structure type and shape of mature bulb of 1,800 accessions	To provide passport and phenotypic information through the EURISCO catalogue
3	Identification of unique and duplicated accessions	To efficiently conserve and provide access to unique germplasm
4	GWAS analysis	To promote use of PGRFA
5	Cryopreservation training	To efficiently conserve and provide access to unique germplasm
6	Development of a cryopreservation strategy	To efficiently conserve and provide access to unique germplasm
7	Development of a garlic conservation strategy	To efficiently maintain and provide access to unique germplasm

Workplan for the proposed period of the Activity

Brief description of meetings and/or main actions of the Activity.

	Type of Action *(indicate if "meeting" or "other action")*
1	First Online meetings (1. Month) ● Agreement about the planned schedule ● Agreement about delivered material, protocol exchange to collect leaf material and to conduct phenotyping Second Online meeting (6-7th month) ● Preliminary data are used to discuss about threshold levels for number of unique alleles and duplicates ● Identification of unique accessions and duplicates Third Online meeting (12-14th month) ● First sequencing can be used for decision making processes ● Cryopreservation group will be involved to talk about first concepts Fourth Online meeting (22-24th month) ● Data publication, GWAS, and manual development ● Cryopreservation group will be involved to discuss cryopreservation strategy
2	Sequencing (2nd – 9th month)
3	Phenotyping (April – December 2023 and April – September 2024)
4	Data upload (December 2023 and 2024)
5	Cryopreservation training (October - December 2024)
6	Meeting: Final discussion about cryopreservation and conservation strategy (April 2025)

Additional remarks

Indicate any additional remark(s) that is/are important for the evaluation/implementation of the proposed Activity

Remarks:

References

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