ECPGR Activity Grant Scheme

Sixth Call – Phase X (2019-2023)

Final Technical Report

Activity	
TITLE of SUB-AGREEMENT	6th call Grant Scheme Activities
SUB-AGREEMENT NUMBER	L23ROM175
IMPLEMENTING PARTNER	Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK)
AUTHOR OF THE REPORT	Manuela Nagel
DATE SUBMITTED	30th September 2025
REPORTING DATES	18/08/2023 – 31/08/2025
TYPE OF REPORT	Final Technical Report
ACTIVITY 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)
ABSTRACT	Garlic is an important crop in Europe, with about 3,200 accessions maintained in European field genebanks. The aim of the activity was 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. Major achievements during the reporting period are: (1) **GBS data of 2,434 garlic accessions** from 16 European and one international genebanks are available. (2) European partners have **provided phenotypic data for 1,731 garlic accessions**, which will be utilized for genetic analysis and to inform breeding programs. (3) 20 scientists were introduced to **garlic cryopreservation**, and 4 Partners received comprehensive training, enhancing the capacity to cryopreserve European garlic accessions. (4) Discussions on methods and procedures were conducted in **three online meetings and one in-person meeting**, fostering collaboration and knowledge sharing among European partners. In future, two new reference genomes will be produced by April 2026 to fully analyze the GBS data and provide a more comprehensive understanding of the genetic diversity of European garlic. By integrating this knowledge, we aim to **prioritize important genotypes** for cryopreservation, to **create a European garlic core collection** with a centralized access point for garlic breeders and researchers and to **develop a comprehensive conservation strategy**, including cryopreservation, ensuring the long-term conservation and utilization of European garlic accessions.
KEYWORDS	European garlic collection, garlic, Allium, genotyping, cryopreservation

Activity Partners (ECPGR-funded)

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4	Terhi Suojala-Ahlfors	Natural Resources Institute Finland (Luke)	Finland
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Self-funded partners

Partner No.	Name and Surname	Institute	Country
17	Stephan Weise	IPK / EURISCO	Germany
18	Domingo José Rios Mesa, Desirée Afonso Morales	Center for Conservation of Agrobiodiversity of Tenerife (CCBAT)	Spain
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21	Maarten van Zonneveld	World Vegetable Center	International

Research project background

About 3,200 vegetatively propagated accessions of garlic are preserved in European genebanks. Most of the garlic accessions are maintained in field collections and require annual replantation which is time-consuming, costly and risky. Therefore, the safety duplication in different field sites, in in vitro slow-growth storage or by cryopreservation is an essential strategy to conserve the valuable genebank material in the long-term. To develop a comprehensive conservation strategy for garlic genetic resources, we genotyped 2,270 accessions of European garlic collections. Further analysis will identify unique and duplicated accessions which will be prioritized for cryopreservation and for phenotyping in the frame of a core collection. The results of the project will lead to a comprehensive garlic conservation strategy.

Milestones achieved and further activities

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

	Type of Action *(indicate if "meeting" or "other action")*
1	First Online meetings (10th March 2023) ● Agreement about the planned schedule ● Agreement about delivered material, protocol exchange to collect leaf material and to conduct phenotyping Second Online meeting (22nd September 2023) ● Preliminary data are used to discuss about threshold levels for number of unique alleles and duplicates Third Online meeting (18th September 2024) ● First sequencing results were used discuss further potential duplicates ● Introduction to cryopreservation and cryopreservation training Fourth in-person meeting in Gatersleben (20th-22nd May 2025) ● Allium Symposium including scientific presentations ● Preliminary data used to discuss duplicates and plasticity of phenotypes ● Cryopreservation training Fifth Online meeting (planned for July 2026) ● Based on available GBS data, development of a conservation strategy
2	Sequencing, GBS data of **2,434 garlic accessions** are available
3	Phenotyping, data from 1,731 garlic accessions are available
4	Data upload, planned for July 2026 (after the fifth online meeting)
5	Cryopreservation, 20 persons were introduced to garlic cryopreservation at the Gatersleben Allium Symposium (20-22 May 2025); 4 Garli-CCS partners received a comprehensive training at CARC, Prague (17-20 September 2024)
6	Meeting: Final discussion about cryopreservation and conservation strategy, planned for July 2026

Activities during reporting period

1. WP Sequencing

   For sequencing, 1,664 European (non-duplicated) accessions were selected and leaf material was collected by **Partners 2-13, 15, 16, 18-20** between April and June 2023. The DNA was extracted and submitted for sequencing in January 2024 by **Partner 1**. For ease of management, all material received a barcode and a unique project identifier (GarliCCS number) from **Partner 17**. The first GBS sequencing runs were completed on 23rd April 2024. Due to the asynchronous growth period of garlic in Taiwan, **Partner 21** shipped 164 accessions to IPK on 2nd April. In addition, a second GBS sequencing was conducted in August 2024, which included additional replicates of initially insufficient leaf quality (>200 accessions). As a result, GBS data from **2,434 garlic accessions** (2,493 samples including technical reps) from **15 European and one international genebanks** are now stored in the IPK Laboratory Information and Management System (LIMS) and are currently being analyzed by **Partner 1 (Table 1).**

Table 1. Available GBS data from Garli-CCS Partners including technical replicates.

Partner ID	Garli-CCS Partner	Available GBS data
1	IPK, Germany	550
2	LatHort, Latvia	91
3	CARC, Czech RepIublic	596
4	Luke, Finland	7
5	EMU, Estonia	6
6	INRAE, France	108
7	LAMMC, Lithuania	56
8	KIS, Slovenia	50
9	IPGRB Elgo-Dimitra, Greece	28
10	IPTPO, Croatia	116
11	INIAV, Portugal	65
12	IRIAF-CIAF, Spain	323
13	DiCEM, Italy	50
15	INHORT, Poland	279
16	SLU, Sweden	4
21	WorldVeg, Taiwan	164
	Total	2,493

Of the 2,493 DNA samples, 122 million SNPs were generated and distributed across the eight garlic chromosomes. Preliminary phylogenetic analysis, principal component analysis (PCA), and model-based clustering analysis revealed six clusters that correlate with the six infraspecific *Allium sativum* groups: Longicuspis, Pekinense, Ophioscorodon, Subtropical, Sativum bolting, and Sativum non-bolting (**Figure 1**). A preliminary Identity-by-State (IBS) analysis identified approximately 16% of the total samples as potential duplicates. However, further analysis is required to clarify potential duplication within and between the different genebanks.

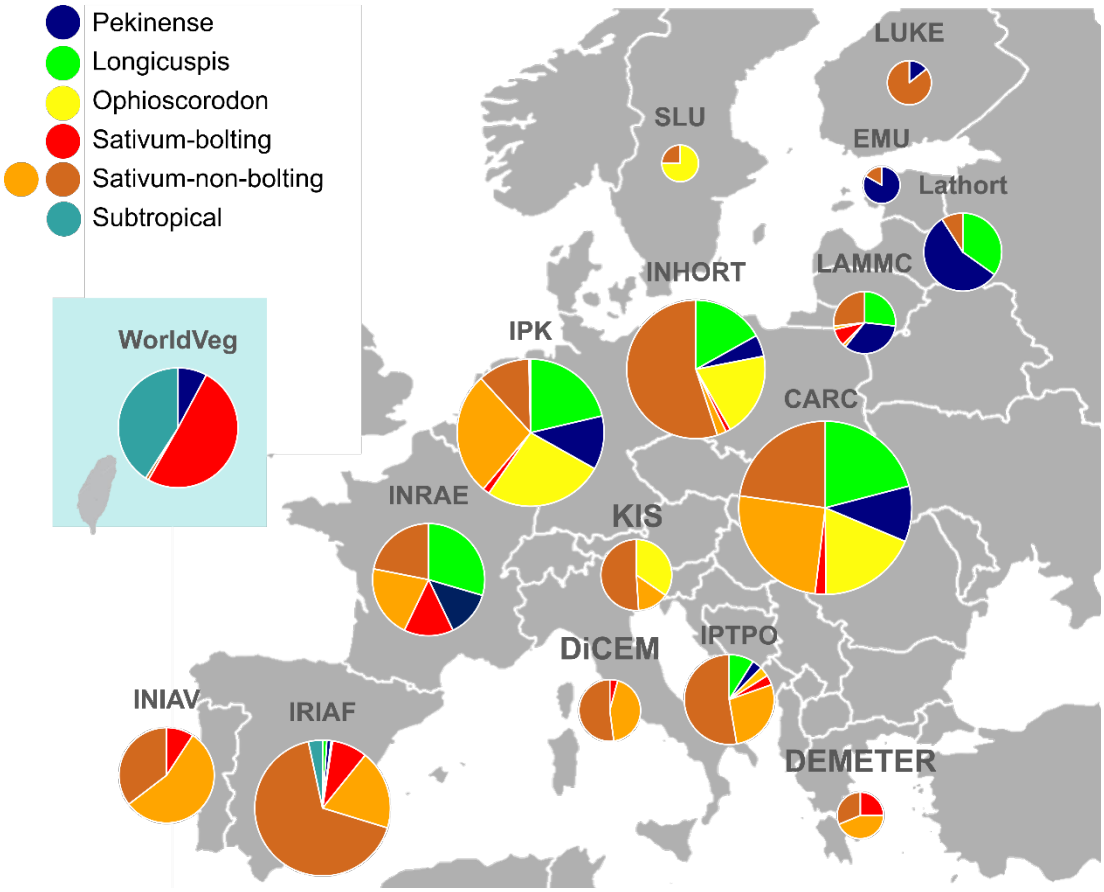

Figure 1. Distribution of infraspecific Allium sativum *groups across genebanks of Garli-CCS partners based on a preliminary analysis of GBS data.. Colour code is given in the figure. Sativum-non-bolting was divided into two further sub-groups and, therefore, received two colours. Abbreviations as in Table 1.*

2. WP Phenotyping

   Descriptors for phenotyping have been agreed upon, and a data collection template was developed by **Partner 17** and distributed to all partners. Phenotyping was conducted by **all European partners** over one or two seasons, from April 2023 to September 2024. Data were collected from all partners. To facilitate the upload to EURISCO, the data were directly imported into an Oracle database. After another final review, the data will be available for further analysis and upload.

3. WP Data accessibility

   Partner 17 is fully involved in the data management and provides GarliCCS numbers for each accession. After data evaluation and verification, genotyping data will be uploaded to EMBL-EVA public repository. Phenotypic data and any missing passport data can then be provided to EURISCO. The consent of the responsible National Inventory Focal Points will be obtained for this.

4. WP Cryopreservation training

   Partner 14, together with **Partner 1** organized a cryopreservation training in Prague from 16th to 20th September 2024. An additional cryopreservation training was organized by **Partner 1** during the final meeting in Gatersleben from 20th to 22nd May 2025. Discussions on a cryopreservation strategy were initiated during the workshops. However, it was agreed that a

cryopreservation strategy can be only part of the conservation strategy and need to be developed together with the curators.

5. WP Garlic conservation strategy
   Three online meetings were held on 10th March 2023, 22nd September 2023, and 18th September 2024, and one in-person meeting was organized by **Partner 1** in Gatersleben, Germany from 20th to 22nd May 2025. The main topics of the online discussions were the organization of the experiments. Additionally, Julie Sardos from Bioversity, Montpellier, France, presented on determinants for duplicate identification in banana on 22nd September 2023, and Bart Panis from Bioversity, Leuven, Belgium, gave an overview of the history and principles of plant cryopreservation on 18th September 2024. The fourth Garli-CCS meeting in Gatersleben was embedded in the Gatersleben Allium Symposium, featuring presentations on garlic production, conservation, and scientific experiments. **All partners** participated in the discussions at the fourth meeting. However, the final discussion on a garlic conservation strategy was postponed until the genotyping data are fully analyzed.

Next steps to be taken

1. WP Sequencing: GBS data are available from **Partners 1-13, 15, 16, 18-20**. However, the available reference genome is not fully satisfactory for mapping the GBS data of garlic diversity. To address this, **Partner 1** initiated a whole genome sequencing project for two accessions, one from the Ophioscorodon group and one from the Longicuspis group. The additional sequences are expected to be available in April 2026 and will support the final and comprehensive GBS analyses which is expected to be finalized by July 2026.
2. WP Phenotyping: Phenotyping data were provided by **Partners 2-13, 15, 16, 18-20**. After integration of updates from **Partner 9, 10, 15** and additional data from **Partner 1**, phenotyping data will be analyzed together with genotyping data and uploaded to EURISCO.
3. WP Data accessibility: Available data will be evaluated, verified and uploaded at respective platforms, latest by July 2026.
4. WP Cryopreservation training: Trainings were successfully finalized.
5. WP Conservation strategy: Information on potential duplicates will be send and to all partners in September 2025. However, final discussion on a garlic conservation strategy was postponed until the genotyping data are fully analyzed, most likely until April-July 2026.