

ECPGR Activity Grant Scheme Proposal Form

First Call – Phase XI (2024–2028)

Activity Proposal

Activity	
Full title	Implementing a trans-EUROpean PEPper LANDrace collection for resilient agriculture
Acronym (or short title)	EUROPEPLAND
Duration of Activity (in months)	24
Start date – End date <i>Please indicate start date not earlier than 3 months after deadline of Call</i>	1 September 2024 – 31 August 2026

Applying Working Group(s)

	Working Group	Indicate name and surname of Working Group Chair
1.	Solanaceae	Pasquale Tripodi

Activity Coordinator

Activity Coordinator	
Name and Surname	Pasquale Tripodi
Working Group	Solanaceae
Nationality	Italian
Current position	Research Director
Institute	Council for Agricultural Research and Economics, Research Centre for Vegetable and Ornamental Crops
Country	Italy
Telephone	089 386217
Email	pasquale.tripodi@crea.gov.it

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	Pasquale Tripodi	Research Centre for Vegetable and Ornamental Crops (CREA OF)	Italy
2	Ifigeneia Mellidou	Hellenic Agricultural Organization – DEMETER (IPGRB, ELGO)	Greece
3	Laura Toppino	Research Centre for Genomics and Bioinformatics (CREA-GB)	Italy
4	Roberto Lo Scalzo	Research Centre for Engineering and Agro-Food Processing (CREA-IT)	Italy
5	Helena Stavelikova	Crop Research Institute, Genetic Resources for Vegetables, Medicinal and Special Plants (CRI)	Czech Republic
6	Ulrike Lohwasser	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)	Germany
7	Adrian Rodriguez Burruezo	Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) - Universitat Politècnica de València (UPV)	Spain
8	Veselina Banova Masheva	Institute of Plant Genetic Resources – Sadovo (IPGR)	Bulgaria
9	Sonja Ivanovska	International Cooperation Institute of Agriculture-Skopje Bul Aleksandar Makedonsk	North Macedonia
10	Willem van Dooijeweert	Centre for Genetic Resources the Netherlands (CGN)	Netherlands
11	Dario Danojević	Institute of Field and Vegetable Crops (IFVCNS)	Serbia
12	Rosella Giunta	CNR-Institute of Biosciences and Bioresources (IBBR) – Headquarter Bari	Italy
13	Veronique Lefebvre	Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE)	France
14	Petre Marian Brezeanu	Research and Development Station for Vegetables Bacau (SCDL)	Romania
15	Filomena Rocha	Banco Português de Germoplasma Vegetal (INIAV)	Portugal
16	Teodoro Cardi	CNR-Institute of Biosciences and Bioresources (IBBR) – Division of Portici	Italy

Self-funded partners

Partner No.	Name and Surname	Institute	Country
1	Gabriella Sonnante	CNR-Institute of Biosciences and Bioresources (IBBR) Headquarter Bari	Italy
2	Karuine Sarikyan	Scientific Center of Vegetable and Industrial Crops (SCVIC)	Armenia
3	Sandra Goritschnig	ECPGR Secretariat, EVA coordinator	Italy

Description of Activity (suggested max. 1000 words)

Please address the following aspects:

– Background:

Food supply in the context of population growth, increased competition for land and water resources and global climate change are among the most important challenges for agriculture nowadays. The Agenda for Sustainable Development and the European Green Deal require raising production while limiting overexploitation of natural resources. While more resilient crops need to be developed to provide food and agricultural products in a sustainable manner, the liberal use of high inputs has resulted in the loss of genetic diversity and the stagnation of yields for crops in less favorable areas. Increasingly landraces (LRs), i.e. locally adapted traditional varieties, are being replaced by modern cultivars less resilient to abiotic stresses and thereby losing a valuable source of germplasm for meeting the future needs of sustainable agriculture. LRs are important not only for intensive agriculture and large-scale retail trade, but also for small farmers in rural areas, sustaining local economies. The Mediterranean basin of Europe is a Centre of secondary diversification for pepper, thus rich in highly diverse landraces in terms of plant and fruit types. LRs are crucial for resilient agriculture, thanks to their broad adaptation to contrasting environments. Their genetic heritage represents a reservoir of genes that can be exploited for the development of new varieties through classical and advanced breeding and genetics methods. Currently some of the limiting factors in the use of LR *ex situ* collections are (i) missing or incomplete passport data, and (ii) the precise characterization of the collections. The advent of next generation genotyping technologies may provide new and reliable information for the description and optimization of LR collections bridging existing knowledge gaps. This project aims to improve both phenotypic descriptors and genomic characterization of *Capsicum* local landraces stored in the main European genebanks and research institutions making them available through AEGIS and the EVA Pepper network and implementing the new information in EURISCO.

– Justification:

The proposed activity aims to build a novel core collection of local pepper varieties depicting the diversity existing in Europe, through a synergic collaboration among ECPGR Solanaceae WG members. Core collections are essential to explore the genomic variation of germplasm resources and identify candidate genes through genome wide association mapping approaches. To that end the LR collection will be assessed with main crop phenotyping descriptors and genotyped using Next-generation sequencing (NGS) methods. Descriptors and phenotyping data will be available through the EURISCO database, providing access to information to the community, thus bolstering the information system for plant genetic resources. Genotyping data will expand the existing genomic

information developed within the EVA pepper project. To this end, the same genotyping technology will be used to combine the data of the two collections. This approach enhances the valorization of European genetic resources promoting their use for breeding purposes facilitating the use of pepper germplasm for sustainable use. Considering the importance of including all germplasm into the AEGIS system, a strategy for safety duplication across partners will be established also considering the presence of the main European genebanks in the consortium. The activities of EUROPEPLAND will meet the priority targets (P1) of the ECPGR Phase XI (Table 3, Target 3; Table 4, Target 3; Table 5, Target 1).

– *Rationale for the choice of partners:*

All project partners are members of the WG Solanaceae and represent main genebank curators in their respective countries by holding hundreds of local pepper landraces. All partners have matured skill and competences for the conservation and characterization of *Capsicum* genetic resources and therefore are able to provide germplasm material along with related information (descriptors, phenotyping). Furthermore, the network encloses expertise in genomics and phenotyping for metabolites and abiotic stresses. Several partners have been involved in national and international projects aimed at the conservation and valorization of the genetic resources of pepper (e.g., G2P-SOL, PRO-GRACE), enhancement of sustainability of crops in organic agriculture (e.g., BRESOV, LIVESEED, LIVESEEDING), and promotion of a plant genetic resources community for Europe (PRO-GRACE) and some are part of the existing EVA pepper network. Overall, 19 partners from 13 different European countries (**Figure 1**) will join the activities of EUROPEPLAND ensuring the possibility of representing the entire variability of landraces on the continent.

Partners from Central and Western Europe are represented by four major genebanks (INRAE, INIAV, CGN and IPK) holding hundreds of LR accessions including pepper local landraces selected in Portugal and France. Similarly, partners from Southern Europe are represented by two main genebanks (CNR and COMAV-UPV) and research institutes holding local pepper varieties from Italy and Greece. Furthermore, CREA and CNR partners from Italy have specific expertise in genomics and bioinformatics and platforms for the assay of metabolites and salt/drought stresses. The germplasm from Eastern Europe, which represents an important pool of diversity, in particular for the mild and spicy forms of paprika, is represented by six partners holding hundreds of ancient pepper cultivars. All partners have the possibility to grow and multiply the accessions in their own greenhouses and experimental fields facilities.

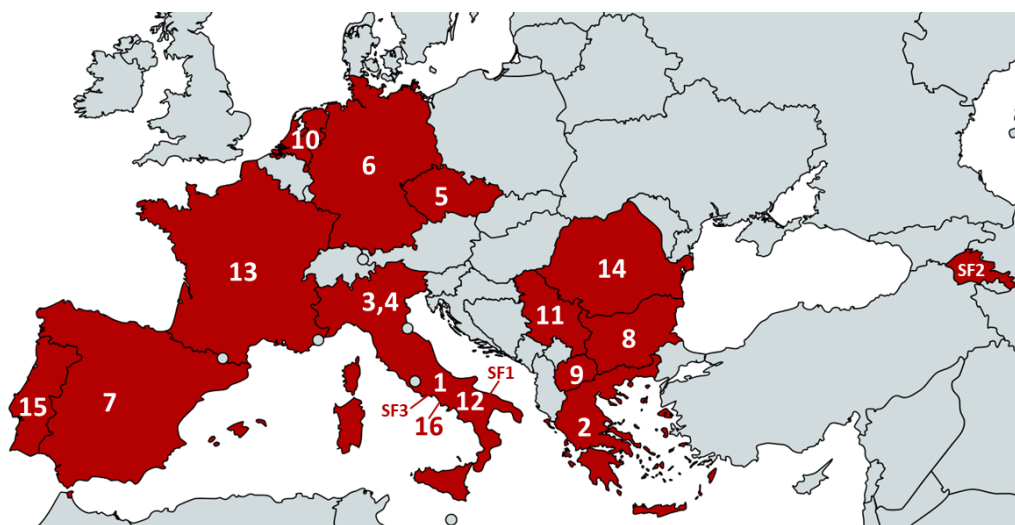


Figure 1: Partnership involved in the EUROPEPLAND proposal. Numbers indicate the ID reference for each partner. SF indicates self-funded partners.

– *Methodology or Approach:*

A list of the available genetic materials with focus on the domesticated pepper species *Capsicum annuum* will be provided by each partner along with existing passport data. The overall strategy (Figure 2 and Figure 3) will include several tasks:

Task 1 – Core collection development (M1-M6)

A core collection of about 300 individuals representing the whole of European diversity will be established. The objective will be to define a core collection as diverse and representative as possible. The criteria for selection will be based on the origin (collecting site) of LRs, main fruit characteristics (e.g., fruit size, shape, color) and qualitative features (sweet or hot peppers). Regeneration of seeds of the selected accessions in view of possible further exploitations (e.g., EVA consortium) will be carried out by each providing partner. To that end, the seed amount will be defined considering also any requirement for phytosanitary tests. A safety storage of the entire core collection will be also defined involving one or more genebanks.

Task 2 – Phenotyping and genotyping (M6-M18)

A common protocol for descriptors and main phenotyping data to be collected on the core collection will be established. The protocol will follow the descriptors already used in the EVA pepper network (Self-Funded P3) in order to incorporate data of the two collections. Each WG partner responsible for the accessions of the core collection will characterize germplasm according to the guidelines. Analysis of specific bioactive compounds able to confer taste and flavor profile of interest for the consumers (P4) and evaluation of salt stress tolerance (P16) will be performed in selected genotypes. Genomic activities will be centralized by the coordination partner (P1): lyophilized leaves, or seeds (under sMTA agreement) will be shipped for nucleic acid isolation and plate preparation. Genotyping will be performed in service using the existing 19K pepper array available at SGS Trait Genetics. This technology has been used to genotype the EVA pepper collection.

Task 3 – Data analysis and integration (M19-M24)

Analysis of genomic data and integration with passport and phenomic data will be performed. Analysis will include the investigation of genetic diversity and population structure, phylogenetic relationships of accessions, level of similarity and/or possible redundancy, attempt for candidate gene identification. Based on genomic and phenotypic diversity few accessions (<10) will be selected for abiotic stress and metabolic trials as a proof-of-concept for the potentiality of LRs for further sustainable and quality-based breeding. Germplasm will be linked to specific DOI and included in AEGIS, while phenotypic data will be uploaded on EURISCO database, genotypic data in Zenodo. Data will be also linked to the EVA tools: data collection templates and phenotypic database.

Task 4 – Meetings and dissemination (M1-M24)

Three virtual meetings, Kick Off (M1), Interim (M12) and Final (M24), will be organized to define the workflow of the project, the progress and final discussion and exploitation or results including dissemination. A possible in-person meeting could be organized in conjunction with scientific conferences (e.g., EUCARPIA) or project meetings (e.g., EVA pepper). Main results will be disseminated in conferences and/or in peer review journals. The developed materials will also feed into evaluation activities of the EVA Pepper network.

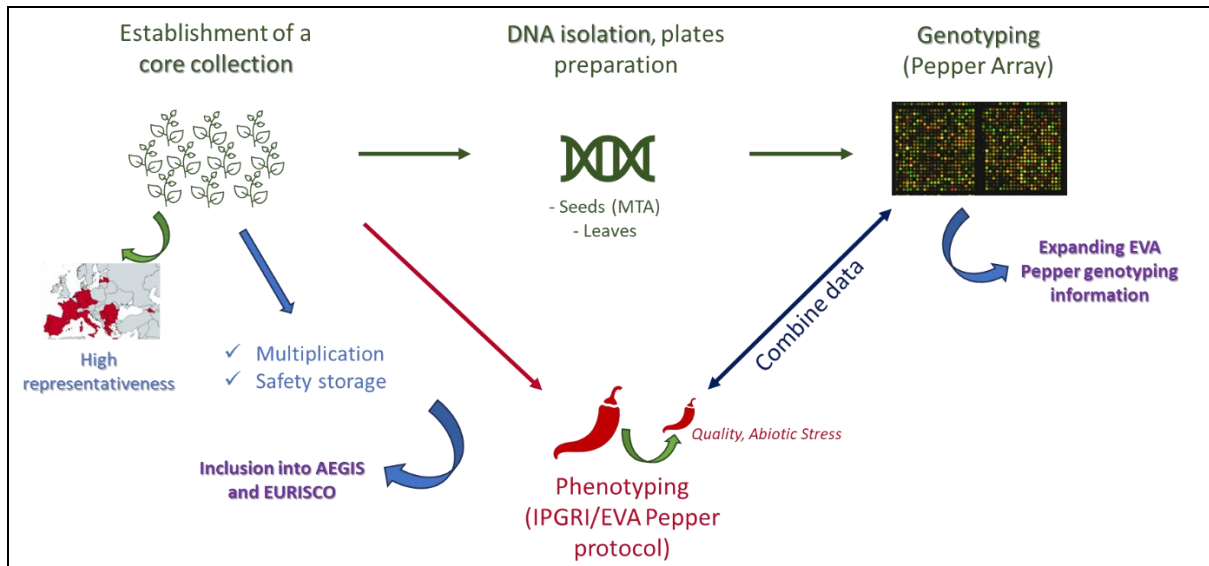


Figure 2: Diagram of project activities. A highly representative collection of the diversity existing in Europe is defined. Each partner will perform phenotyping and multiplication activities. Leaf samples or seeds will be sent to the partner ID1 for DNA isolation and plate preparation. Genotyping will be performed with the available pepper array and data combined.

Task	Year	2024				2025								2026												
		9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
T1	Core collection establishment	█	█	█	█																					
T2	Seeds/Leaf exchange					█	█																			
T2	DNA extraction						█	█	█	█																
T2	Genotyping											█	█	█	█											
T2	Plant grown and phenotyping						█	█	█	█	█	█	█	█												
T3	Data integration/analysis																				█	█	█	█	█	█
T4	Meetings		█	█																					█	█

Figure 3: Gantt chart of EUROPEPLAND project proposal

– Description of genetic material:

The genetic material will be provided by the institutions involved in the consortium. In total approximately a thousand landrace accessions of *C. annuum* are maintained by partners. At CREA (P1 and P3) over 80 accessions representing the variability existing in the Italian territory are stored, among these several PGI/PDO (Protected geographical indication/Protected designation of origin) genotypes are available. CNR IBBR (P12 and Self-Funded P1), the main Italian genebank holds over 60 accessions retrieved from Southern Italy and Albania. IPGRB, ELGO (P2) can share 20 accessions from Northern and Southern Greece (from a collection of > 140 traditional varieties maintained in the Greek genebank) while COMAV (P7), the main Spanish genebank stores 40 landraces representative of the entire territory, including several PDOs/PGIS.

Partners from Eastern Europe will provide over 120 ancient varieties, 25 from SCVIC (Self-Funded P2) representing different regions (Ararat, Armavir, Tavush, Vayk marz, Mecamor, Echmiadzin) of Armenia, 13 from IFVCNS (P11) retrieved from Northern, Central and Southern Serbia, 38 from CRI (P5) representing the variability of Southern Czech Republic, 20 from IPGR (P8) retrieved from

North, Central and Eastern Bulgaria, 20 from SCDL (P14) retrieved from diverse areas of Romania, about 10 from North Macedonia (P9).

The remaining Western and Central European partners are major genebanks holding thousands of accessions of *Capsicum*. Among these, CGN (P10) maintains 93 *C. annuum* landraces retrieved from Spain (93%) and Hungary and a similar number of landraces with different origins is stored at IPK (P6). INRAE (P13) maintains about 50 accessions, representing the French landrace collection, while INIAV (P15) will provide about 20 accessions from Portugal. The core collection will not overlap with materials already included in the EVA pepper collection.

All material encloses both sweet and hot types with different level of pungency, all types of size (small, medium, large) and shape (elongate, almost round, triangular, campanulate, block), fruit colour at immature stage from white to dark green turning from yellow, orange, and red at maturity. Wax/white type will be also included.

In this way the partnership can cover the entire variability of local pepper varieties present in Europe.

– Expected impact.

The EUROPEPLAND project encourages the creation of a network of researchers and genebank managers aiming at the enhancement of the catalogue of information of European local varieties by creating a comprehensive collection, providing phenotyping information, and deeply assessing the genetic diversity. Additionally, the molecular characterization of the collection will allow to define geographical based genomics footprints providing a more rational management and conservation of accessions through the identification of specific subpopulations and accessions with high level of redundancy or similarities. The construction of core collections containing a large part of the genetic and phenotypic diversity will allow users to screen the diversity and perform candidate gene identification via association mapping. Therefore, the collection will be powerful for research and breeding purposes. Through engaging with the private sector stakeholders in the EVA Pepper network this collection will become immediately available to users and the integration into AEGIS will ensure the long-term conservation and availability of this important resource. In this large framework, the project will improve the value of food chain for local products of high quality with concrete and positive impacts on the end-users.

– Links with other non-ECPGR projects or individuals:

The project is linked to: i) G2PSOL - Linking genetics resources, genomes and phenotypes of solanaceous crops (2016-2021), aiming at harnessing and utilizing the genetic diversity of major Solanaceae genetic resources as key to the sustainability of agriculture in the face of a changing environment and the appearance of new pests; ii) PRO-GRACE - Promoting a Plant Genetic Resources Community for Europe (2023 – 2025) aiming at the development of an European research infrastructure dedicated to cataloguing, describing, safeguarding and enhancing European plant genetic resources for food and agriculture; iii) ECPGR EVA-PEPPER (2020 – ongoing) building a network of researchers across Europe evaluating genetic resource of pepper through field phenotyping, disease resistance trials, SNP genotyping; iv) LIVESEED (HORIZON 2020, 2017-2021) and v), LIVESEEDING (HORIZON EUROPE, 2022-26), aimed at diversifying, improving and boosting the use of organic seeds towards a more diverse and resilient agri-food system in Europe.

Expected products and related ECPGR Priority activity.

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 contribution to <i>Plant Genetic Resources Strategy for Europe</i> target
1	List of accessions to include in the new core collection, DOI assignment, characterization, and seed multiplication.	Consolidating and sustaining <i>ex situ</i> conservation: P1 Support to countries to identify and include material into AEGIS, including regeneration and safety-duplication.
2	Phenotypic assessment of the collection using a common protocol that includes international descriptors defined for <i>Capsicum</i> spp.	Strengthening a comprehensive information system for plant genetic resources for food and agriculture (Documentation): P1 Compilation and transfer of existing Characterization and Evaluation data to EURISCO.
3	Genotyping information of the core collection with the pepper SNP array	Promoting sustainable use of PGRs: P1 Continuation of existing EVA Networks.
4	A new core collection, phenotyping information and possible additional partners to include in the ECPGR EVA Pepper	Promoting sustainable use of PGRs: P1 Continuation of existing EVA Networks.

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	<p><i>Meetings:</i></p> <ol style="list-style-type: none"> 1. A virtual kick-off meeting to discuss: <ul style="list-style-type: none"> - Germplasm accessions to share and their status in national/international genebanks - Information of germplasm in EURISCO and activities to perform for inclusion in AEGIS - Defining a common protocol for descriptors and phenotyping to be used by each partner, building on EVA Pepper tools. - Defining protocol for seed regeneration and safety duplication and storage - Phenotyping activities related to quality and abiotic stress, also involving EVA Pepper network. - Actions to perform for genotyping the core collection. 2. An interim virtual meeting to discuss progress of activities and sharing first results. 3. A final virtual meeting to discuss: <ul style="list-style-type: none"> - Discussion on gathered data and collected samples - Status of the information of the collection in EURISCO and AEGIS databases - Status of seed availability and their safety storage - Program for further activities (also within EVA pepper network) <p>* Either meeting 1, 2 or 3 could be performed in hybrid mode (virtual and in person if they fall in the same period of other meetings such as conferences or EVA pepper meeting)</p>
2	<p><i>Other action:</i></p> <p>Germplasm selection: identification of pepper landraces accessions for building the trans-European core collection.</p>
3	<p><i>Other action:</i></p> <p>Characterization with plant descriptors and phenotyping protocol of the core collection and regeneration of accessions</p>
4	<p><i>Other action:</i></p> <p>Genomic DNA isolation and genotyping. Analysis of data (genomic diversity, population structure) and integration with phenotyping characterization</p>
5	<p><i>Other action:</i></p> <p>Application of DOI to individuals of the core collection, upload of the information in EURISCO, regeneration of the core collection and inclusion in AEGIS for promoting its use.</p>

Additional remarks

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

Remarks:

In the past decades, great efforts have been made in the recovery and storage of plant genetic resources in genebanks. However, genomics applied to PGR *ex-situ* is still lacking and very few core collections, representing as much as possible the existing diversity, have been established. Landraces hold the ability to adapt to less favorable growing conditions, having been selected over time within environments characterized by pest and disease pressure and with reduced fertilizer and pesticides inputs. For pepper, several landraces are present in genebanks and research institutions, but no extensively characterized core collection has been released. The possibility to exploit this resource provides a valuable opportunity for both PGR efficient management and identification of useful alleles to face the agricultural challenges of the coming years. The project is in accordance with objectives and targets of the PGR strategy for consolidation and strengthening of *ex situ* conservation and for the obtainment and transfer of related data to public databases, thus ensuring the availability for promoting the sustainable use.

**Please send the completed form together with the budget table to the
Chair of the submitting Working Group for submission of the Activity proposal.**