





Federal Ministry of Food and Agriculture

# Summary report of the

# Annual meeting of the European Evaluation Network (EVA) for Pepper and EuroPepLand kick-off meeting

30-31 October 2024 El Ejido, Spain



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The 2024 annual meeting of the EVA Pepper network was organized jointly with the kick-off meeting of the ECPGR Grant Scheme activity EuroPepLand and took place from 30-31 October 2024 in El Ejido, Spain, co-organized with Semillas Fito. The meeting was organized in a hybrid format and attended by 16 participants in person and 11 online. The agenda of the meeting is attached as Appendix 1 and the list of participants as Appendix 2.

## 1. Introduction

The EVA coordinator Sandra Goritschnig opened the meeting, welcoming project partners attending in person and online. She noted the goal of the meeting, which was to review the results of the EVA Pepper network and discuss their dissemination as well as options for continuing joint activities after the end of the current project, and how it could link with EuroPepLand. Combining the two projects in one meeting provided a good opportunity to connect their work, with the expectation that EuroPepLand activities would feed into the EVA Pepper network, creating important synergies. Marta Fernandes Zurro from Semillas Fito welcomed meeting participants in El Ejido highlighting the importance of pepper breeding and cultivation for the region of Almeria, which is one of the main producers and exporters of peppers in Europe with a greenhouse area of approximately 33,000ha and a growing season from August to March.

# 2. Results from the EVA Pepper project

### 2.1 Overview of the current status of the ECPGR Evaluation Network EVA

After a round of introductions, S. Goritschnig recalled the framework of the EVA network and its guiding principles and updated participants on developments within the EVA Pepper and other networks. The current EVA project which has funded the EVA Pepper network so far, has been running since 2019 and is ending in December 2024. Since all evaluation activities have been finished, the remaining time should be dedicated to collecting any missing datasets and analyzing them for dissemination and further exploitation. With the integration of the EVA networks as an ECPGR initiative in Phase XI, funds from the regular budget are now available to continue coordination of the EVA networks and its in-kind activities, while specific activities that require funding should be supported through additional projects, such as EuroPepLand. EVA Network partners have disseminated results in various conferences, including the Eucarpia General Congress in Leipzig, Germany and the 66<sup>th</sup> SIGA Congress in Bologna, Italy, where presentations on our work have been received with great interest. Partners were invited to follow ECPGR on Linkedin and to share news and media, such as the EVA promotional video, which was published in early 2024.

The EVA networks are being extended through two ECPGR Grant Scheme activities, MALANIRS (EVA Maize and Maize Working Groups) and EuroPepLand (EVA Pepper and Solanaceae Working Groups), providing additional collections from new partner genebanks and some funding for evaluation activities. Furthermore, a new 3-year project 'EVA Boost', again funded by Germany, provides funding to implement the new EVA Legumes network and to prepare a possible EVA Perennials network on fruit trees and berries.

Updating on recent developments in relation to Tomato Brown Rugose Fruit Virus (ToBRFV), this pathogen is now present all over Europe and is considered an A2 pest. The updated EU regulation and Pest Risk Analysis by EPPO have been shared with the partners on the project SharePoint. It is expected that the emergency measures in place for this pest will expire as of 2025, however, phytosanitary measures are still needed to prevent the spread of the pathogen,

including the use of plant passports, monitoring and relevant plant health precautions during the growth of pepper plants.

The EVA Pepper network currently has 15 partners from 10 countries, including 6 breeding companies. Some of the partners have not been very responsive as of late, at least partially due to changes in the responsibilities of contact persons. The network membership will be reviewed by the EVA Coordinator, also taking into account the invitation to EuroPepLand partners to join the network. This will be implemented through an amendment to the current cooperation agreement.

#### 2.2 Review of network work plan 2019–2024

S. Goritschnig provided an overview and summary of the work plan agreed for the EVA Pepper network. Multiplication activities in 2020 were followed by field, greenhouse and lab evaluations as well as genotyping of ~180 pepper accessions in 2021 to 2023. Thanks to an amendment and extension of the current project, additional genotyping could be done and abiotic stress tests were conducted on 260 pepper accessions; results of these activities were presented in this meeting.

Although the EVA Pepper collection was mainly sourced from CGN, the Netherlands, it represents a great geographic and phenotypic diversity. At this moment, data from 17 trials are available in the EURISCO-EVA database, including two biotic stress trials in the lab. Evaluation data was collected on 181 accessions and 26 traits, with on average eight observations per accession and trait in field trials. Initial analysis showed variation across environments for quantitative traits such as flowering time and plant height. Field trial data should be considered complete, the data for abiotic stress experiments and ToBRFV tests are being finalized, but analyses have already started.

#### 2.3 Preliminary results from additional pepper genotyping

P. Tripodi (CREA) presented updated results from genotyping, which has now been extended to 230 accessions, of which 160 are *Capsicum annuum* and thus available for GWAS analysis. The original EVA collection was enlarged by including diverse *C. annuum* accessions from the G2PSol project and now represents material originating from 48 countries across the globe. Forty-two percent (42%) of the collection are landraces, mostly from outside Europe, and phenotypic data are available for the newly added accessions. Because the high-density 19k SNP array (https://doi.org/10.1038/hortres.2016.36) used for the first set of 161 accessions was no longer available, an updated 10k SNP array developed by SGS Traitgenetics was used to genotype another batch of 80 accessions, including 11 which were repeated to facilitate comparison of the new platforms. The analysis showed that the array, in addition to halving the eliminated SNP markers were associated with Chromosome 00. While the old array produced ~11,000 useable SNPs after quality filtering, the new array provides around 9,000 useable markers evenly spread across the chromosomes, a noticeable improvement.

Admixture analyses identified 10 subpopulations, which reflect the species, origin and biological status of the accessions, including 80 admixed accessions, which could also indicate heterogeneity. This is evidence that the EVA pepper collection is highly diverse, which is a prerequisite for further GWAS. A principal component analysis separated accessions by species and origin and highlighted Europe as a secondary centre of origin for pepper with high diversity observed in European materials, which was also confirmed by analysis of the population differentiation coefficient Fst.

Zooming in on the *C. annuum* accessions, they separated based on origin with a new cluster observed for Eastern European material and showed good diversity for morphological traits. Taken together, the EVA pepper collection is representative of the variation of *Capsicum* (in

terms of species, traits, provenance, etc.) and will be further explored for additional phenotypic traits and in association studies. The smaller and cheaper SNP array covers the pepper genome effectively and is a good tool for genotyping, also within the EuroPepLand project. The collection of European Pepper landraces that will be developed in EuroPepLand will also be useful for EVA and should be integrated into the EVA collection for additional experiments and analyses.

### 2.4 Results from analysis of phenotypic data of abiotic trials

Giorgia Batelli (CNR) presented the results of the abiotic stress trials conducted at CNR in Portici. Salt stress has been selected for this investigation as high soil salinity is a growing problem in agriculture, affecting more than 100 countries or 23% of the total cultivated area. Salt rapidly accumulates in arid or semiarid areas, where water demands are met through irrigation and is due to the use of poor-quality irrigation water. This complex stress is particularly harmful to peppers where 14% of fruit yield loss occurs as a result of each increase in salt level of 1.0dS/m (Abdelaal et al., 2020). The protocol was developed using commercial varieties and some EVA accessions to assess the optimal salt concentrations of the treatments and establish the best traits as proxies of stress tolerance. For the experiment seeds of ~160 pepper accessions (primarily C. annuum) were germinated in polystyrene trays and treatments applied by floating on nutrient solution without or with varying concentrations of NaCl. and traits measured three weeks after the treatment initiated. Morphological traits such as fresh and dry biomass, height, leaf mass and area (using image analysis), SPAD and chlorophyll fluorescence were measured and used to derive stress-related indices. The collection displayed a large variation in salt-induced growth reduction, but there was no clear clustering of the nondomesticated wild pepper species. Principal component analysis (PCA) showed that the first four components explained ~84% of the variability, and PCAs were associated with different traits. Clustering separated the accessions into tolerant, moderately tolerant, moderately sensitive and sensitive groups, and follow-up experiments will be conducted on the tolerant and sensitive accessions to confirm the initial results.

In the discussion, it was noted that the EVA collection had not been selected for abiotic stresses but given the growing importance of salt stress, previous knowledge should be considered for the selection of the EuroPepLand collection. Colleagues from Semillas Fito reported that in the region the quality of irrigation water is a growing problem, with the quality of pepper grown in Almeria varying with the quality of the the water source. Identifying tolerant material could help develop varieties that thrive with more salty water or could be irrigated with de-salted seawater. For the follow-up experiments, it was suggested to conduct ion measurements on the plant material as well as tracking the conductivity of the watering solutions.

## 2.5 Preliminary results from GWAS for abiotic stress

Teodoro Cardi summarized the initial results from GWAS on the abiotic stress traits. The analysis focused on 130 *C. annuum* accessions for which both genotyping and phenotyping data were available. After some filtering and pruning of the genotypic data presented above, ~4,500 SNPs were used in GWAS following the BLINK model based on the previously identified kinship matrix. QQ plots for all traits under control and stress conditions showed generally good association. The GWAS identified several significant marker-trait associations, in some cases the same SNP was significant for highly correlated traits. Some candidate genes, which may affect the trait expression were identified and in some cases, an allelic effect could be established. Existing literature provides some indication of the involvement of candidate genes in stress responses, but this needs to be confirmed through expression analysis and other follow-up experiments.

During the discussion, it was suggested that the genotyping data be associated also with phenotypic data from the field trials to find correlations and patterns. There should be little variation within accessions, as during regeneration and scoring there was some selection

against offtypes and only small numbers of plants were scored in the trials. Furthermore, most experiments were conducted in controlled conditions in glass- or screenhouses, so there would be lower plasticity, and finally, the material tested was mostly stabilized landraces and improved cultivars. It was noted that stochasticity could result in different ranges of expression of traits in different environments, therefore a large number of replicates should be included in each trial. One suggestion was to perform GWAS on the coefficient of variation to confirm the significance of SNPs that should be in robust QTLs. Correlations between biotic and abiotic stresses should also be investigated, and climatic data considered, although most experiments were conducted in greenhouses.

Going forward, partners commented that the availability of well-germinating seeds should be a main priority within the EVA network. Some accessions produce few seeds, and a minimum number of plants for multiplication should be established. Genebanks typically only distribute small amounts of seeds to requests and users have to regenerate them to produce sufficient seed for their experiments. It was agreed that regeneration, especially of reluctant accessions is no easy task but should be centralized to ensure homogeneous conditions during seed production and the seed requirements should be calculated based on the number of planned trials. M. Fernandes noted that Semillas Fito has collected seeds during their trials for some accessions, including also fruits of off types. Labelling off types during regeneration would allow genetic analysis.

Maintaining the EVA collection was another topic of importance for the network, as the generated data would not be very useful without the associated plant material. Manon Bouet (INRAE) noted that INRAE is maintaining the pepper core collection developed by the G2PSol Horizon project, and requested material is distributed with SMTA and against a service fee. Willem v. Dooijeweert commented that CGN could maintain special collections, but this has to be cost-neutral. Storage of the collection in a black-box arrangement could be done, but the seeds would not be available for distribution.

# 3. EuroPepLand introduction and collections

Presentations of the EuroPepland kickoff meeting can be found on the Europepland website.

### 3.1 Introduction to EuroPepland

Chair of the Solanaceae Working Group P. Tripodi presented the concept and rationale of the EuroPepLand project, highlighting the value of genetic diversity in landraces as a resource for breeding cultivars better adapted to diverse environments and more resilient to climate change. As noted, the Mediterranean basin of Europe is a centre of secondary diversification for pepper, however, there is no pepper core collection for European local varieties. The project aims to develop this resource, generate genomics data that will assist genebanks in managing their material, and update/verify existing passport data (Milner et al 2019 Nat Gen, Tripodi et al PNAS, 2021). The partners in EuroPepLand combine pepper accessions adapted to the southeast, south, central and eastern Europe representing the global European diversity of pepper. The project runs until 2026 and builds on or links with several past and ongoing European projects, especially with EVA Pepper, for which it is expected to provide new interesting materials.

#### 3.2 Descriptions of European Pepper landrace collections

The participating genebanks were invited to briefly present their institutes and pepper collections, including any past or ongoing research projects.

<u>CREA-OF</u>, <u>Pontecagnano</u>, <u>Italy</u>. P. Tripodi introduced the institute which has been active in multiple research projects on pepper, including EVA Pepper and G2PSoI, assuming in most of

them activities related to genotyping and breeding research. CREA-OF has a pepper landraces collection of 60 accessions, however, since the institute is not a genebank the seeds are only stored in a climate chamber under controlled conditions.

<u>INRAE-GAFL</u>, Avignon, France. M. Bouet presented their collection which includes 61 French pepper landraces, some without specific names and 23 traditional varieties. The oldest pepper in the catalogue dates back to 1877. Morphological data are available for all accessions and the majority also have been genotyped in different projects. INRAE have been involved in multiple projects on pepper, most notably recently G2PSol for which they are maintaining the core collection of 400 pepper accessions. The accessions are distributed with SMTA and a phytosanitary certificate.

<u>IFVCNS</u>, Novi Sad, Serbia. Dario Danojević presented his institute, which is involved in multiple research projects and breeding activities, having developed several high-yielding and disease-tolerant pepper varieties. Their pepper collection includes breeding lines as well as ~60 traditional varieties including 13 local landraces which will be characterized morphologically and are available with SMTA.

<u>CGN</u>, Wageningen, the Netherlands. W. v. Dooijeweert presented his institute which is the only genebank in the Netherlands, focusing on conservation of vegetables and potato plant genetic resources and currently expanding their collections to include more species and diverse material from various countries. The CGN pepper collection has ~1,000 accessions of global diversity, but no local landraces, which have been largely characterized with available pictures and phenotypic data. Within G2PSol and the Capsicum Genome Initiative, a large part of the collection has been genotyped. Their collection has some Spanish as well as Eastern European landraces, and ongoing collaborations are working on integrating pepper accessions from Albania and Northern Macedonia in the catalogue and making them available for research and breeding, supporting the limited capacities of the holding institutes in these countries. All material in the collection has been tested for ToBRFV and ToMMV and visually inspected during regeneration, and is available for distribution with SMTA and phytosanitary certificates.

<u>SCDL</u>, Bacau, Romania. Andreea Antal presented activities of the Vegetable Research and Development Station, Bacau, which works on conservation, breeding and cultivation of various vegetable crops. In pepper, national projects examined the comparison of field and controlled condition trials. Their pepper collection includes 400 diverse accessions of which 23 were included in a pilot experiment in 2024 to gather phenological and morphological data.

<u>CNR-IBBR, Bari, Italy.</u> Rosella Giunta presented the Mediterranean Germplasm Genebank in Bari, which holds a pepper collection with accessions mostly collected across Italy between 1971 and 2000. Most accessions are currently unavailable and need regeneration before they can be distributed, also some historical materials have been lost entirely. Phenotypic data are available for ~30% of the collection, however they do not use standard descriptors. Fifty-one (51) Italian landraces could be contributed to the project, however, the phytosanitary status of accessions is not determined, making seed sharing more difficult.

<u>CRI, Dept. of Vegetables and Special Crops, Olomouc, Czech Republic.</u> Helena Stavelikova presented the Czech pepper germplasm collection, which was established in 1951 and encompasses 544 accessions from 26 countries, with the majority being open-pollinated varieties from Eastern Europe (Czech Republic and former Czechoslovakia, Hungary, former Soviet Union, Bulgaria and Romania). They multiply 15–20 accessions per year, using 40 plants to retain the diversity within the accession, and characterize them based on IPGRI descriptors. All accessions have been tested negative for ToBRFV. For the EuroPepLand CRI is proposing 38 landraces.

<u>COMAV-UPV</u>, Valencia, Spain. Adrian Rodríguez Burruezo connected online to present the collection and work of COMAV, as he could not travel due to the disastrous flooding in Valencia on 29 October 2024. COMAV was founded in 1999 based on the seed bank of the Universitat

Politecnica de Valencia and works on the conservation and breeding research on various vegetables, including Solanaceae, having developed diverse research lines. Putting the research into an economic context, he highlighted the importance of pepper as a versatile vegetable and spice in different culinary traditions, with a global production of 25 million tonnes per year. Spain produces ~1.5 million tonnes per year, with a value of >1.6 million Euros and an increase in production of 65% over the past 10 years. The production sector is however facing several challenges, from competition by other countries (especially North Africa), for which the diversification of production and introduction of more landraces into the sector would be a way to diversify the diet and contribute to sustainable farming systems, contributing to a more resilient agrifood system. Based on previous research studying the genetic and phenotypic diversity of their collection, and the experience from various extension projects introducing adapted varieties and landraces to local farmers, also under organic conditions, they identified 40 suitable Spanish landraces for the EuroPepLand project, including autochtonous and endemic accessions, representing not only genetic and morphological diversity, but also different responses to growing conditions, stress and quality factors.

Faculty of Agricultural Sciences and Food, Skopje, North Macedonia. Sonja Ivanovska presented her institute which is the oldest agricultural institution in the country. The institute of crop production is involved in various research and breeding activities on plant genetic resources. Since 2014 they have been conducting regular collecting missions to introduce local landraces of different crops into the genebank, which now includes 1,216 pepper accessions, which need to be regenerated. There are currently no research projects and limited capacity in the institute, but a collaboration with CGN since 2020 has enabled the institute to multiply several hundred local pepper accessions which will become available to users through the CGN catalogue. The North Macedonian pepper collection has a large diversity in shapes, colors and uses, with the embroidered peppers representing particularly interesting local varieties.

<u>IPK, Gatersleben, Germany.</u> Ulrike Lohwasser presented their work and pepper collection, which includes 1,015 landraces from 44 countries, but none local to Germany. Between 50 and 60 accessions are regenerated annually, following a specific protocol and collecting characterization data in the process as well as confirming taxonomic identity based on ECPGR minimal descriptors. Plant material is generally available with SMTA (even though pepper is not part of Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture), but only peppers tested negatively during regeneration is available for distribution. The pepper collection of IPK has been genotyped by GBS in the G2PSol project, and could provide some interesting landraces from countries not represented in the current partnership to the EuroPepLand project.

<u>IBPGR ELGO-Dimitra, Thessaloniki, Greece.</u> Ifigeneia Mellidou shared some slides on the activities of her institute on pepper. They have been partners in EVA Pepper and contributed to evaluations of the first set of EVA Pepper accessions and are generally interested in research towards genetic improvement of *C. annuum*. Their small pepper collection includes ten Greek landraces as well as a number of modern cultivars and breeding lines.

<u>SCVIC, Darakert, Armenia.</u> K. Sarikyan shared slides describing her institute which was established in 1949 and is engaged in selection, breeding and seed production of major vegetable crops, especially tomatoes, eggplants and peppers. Past and ongoing research activities explore the adaptation of different pepper accessions to local conditions and their inclusion in breeding programs. Their collection includes a small number of local landraces, both hot and sweet varieties, which could be regenerated for the EuroPepLand project in 2025.

<u>IPGR, Sadovo Bulgaria</u>. Veselina Masheva shared presentation about her institute and collections. The Institute of Plant Genetic Resources (IPGR) in Sadovo is a part of the Agricultural Academy at the Ministry of Agriculture in Bulgaria. It is an academic organization with rich traditions in the area of plant science and research, consisting of two scientific

departments: Plant Genetic Resources and Breeding-Genetics and Variety Maintenance. The National Genebank was established in 1984 with the support of a FAO project to promote conservation of the country's great biodiversity, building a collection of >65,000 samples of 2,670 plant species. The pepper collection of IPGR, Sadovo includes 460 introduced accessions from 27 countries, 1,760 Bulgarian landraces and local populations from expeditions and 47 breeding lines. Per year 30–40 acssessions are propagated, using 20 plants characterized based on IPGRI descriptors. For EuroPepLand, IPGR, Sadovo offers 20 accessions.

## 4. EuroPepLand – Work planning

P. Tripodi reminded the different consecutive tasks of the EuroPepLand project: 1) core collection development, 2) genotyping and phenotyping and 3) data analysis and integration, all accompanied by regular meetings and dissemination activities. Given the interesting diversity presented by the partner genebanks, the core collection should provide a good cross-section of the diversity present in Europe. The plan is to develop a collection of ~400 accessions, with a focus on *C. annuum* from the European region, that are in EURISCO with a biological status 300 (landraces) and have data for several mandatory descriptors available. A template will be shared with partners to list their available material and a final selection will be proposed by P. Tripodi to represent a broad diversity of geographical origins and interesting traits. The selected accessions will be genotyped with the same SNP-array as the EVA collection in Q1 of 2025, and ideally 15 seeds per accession should be sent to CREA Pontecagnano for DNA extraction. The collection should also be regenerated by the holding genebanks during 2025, with the goal to collect phenotypic data especially for fruits and to produce seeds for additional field trials within the EVA network. Phenotyping during regeneration is using scales to estimate the trait expression, since the data will only be collected in one location, additional in-depth phenotyping should follow within the EVA network. The standard protocol developed for EVA Pepper should be used as a reference. Photos should be taken with a standard colour scale and ruler and additional optional traits could be collected by partners at a voluntary basis.

A small subset of the core collection will be subject to additional abiotic stress trials and biochemical analyses, performed by partner CNR Portici and CREA-IT, respectively, with a goal to determine metabolites in control vs. salt-stressed plants. Although it would be nice to investigate the effect of salt stress on fruit quality, preliminary tests are needed to see if it is feasible to complete the plant life cycle under stress in soil-less conditions. The material selected for these experiments (~10 accessions) will be based on diversity, origin (from salty or arid regions) and known tolerance to salt stress (e.g. from the EVA project). The experiment will serve as a proof of concept for a bigger experiment which could be realized within a larger project. A. Rodríguez Burruezo offered to include some accessions from the EuroPepLand collection under drought conditions as part of a trial within the Horizon project Liveseeding.

In order to integrate the data with EVA and EURISCO, all accessions in the core collection should be assigned a digital object identifier (DOI), which will need to be coordinated with the EURISCO national focal points. This would allow improved traceability, visibility in research articles and the possibility to link derived materials.

In terms of analysis, the plan is to investigate the genetic diversity and population structure of the collection, including also a phylogenetic analysis. Associations with phenotypic data would also be interesting and a publication of the project results is planned to offer visibility to the collection.

Two additional virtual meetings are planned to report on progress and discuss next steps. The next should happen around October 2025 to review the first results from genotyping and phenotyping. Bilateral meetings to clarify any issues will be organized as needed.

During the discussion on the work planning, several suggestions were made to improve communications and logistics. The template to collect information on available pepper accessions should include the information whether they are listed in EURISCO already, as well as the minimum information Genus/Accession number/Holding institute WIEWS code, with which it is possible to extract any documented passport information from the EURISCO database. It was also suggested to include any traditional knowledge on the use of the pepper landraces.

Seed exchange should always be done with SMTA and phytosanitary certificate to avoid introducing any pests into a partner's production areas. The accessions selected for salt stress experiments should be included in the seed shipment for genotyping, to avoid the need for extra paperwork. The accessions in the core collection should be multiplied for additional phenotyping by the EVA network, making sure that phytosanitary measures are followed by all partners. Pablo Quijada González also suggested engaging companies in the regenerations of accessions in 2025.

In addition to the selection of accessions for the core collection and discussing logistics and other details of the genotyping and phenotyping activities, it will also be important to establish how data will be centrally collected and the core collection safely stored, ideally through creating duplicates in other locations as recommended by ECPGR. The EURISCO-EVA information system could be used to manage the collected phenotypic data and will facilitate the publication in the EURISCO public database. CGN might be able to offer safety storage of the collection through duplication within their collection, which could make it available to users, if the original holding institutes agree. Alternatively, CGN would be available to store a black box copy of the core collection, but this could not be made available to user requests.

# 5. Outlook – EVA Pepper 2.0

The discussions centred around the integration of EVA Pepper and EuroPepLand and how to adjust the current EVA approach to better reflect partners' priorities and accommodate their capacities. No EVA field trials are planned for 2025 since this year should focus on finalizing the data analysis and exploitation/dissemination of results. At the same time, regeneration activities within EuroPepLand could be supported by EVA companies that have capacity. Given the heterogeneity of pepper landrace accessions, it will be important to increase the number of plants evaluated per accession, even if that reduces the overall number of accessions included in a single trial. The often low germination of some accessions also requires a larger number of seeds to be allocated per trial, for 10 trials with 30 seeds each, a minimum total of 300 seeds will be needed for evaluations and should ideally be freshly regenerated. Regeneration requires advance planning and extra time for distribution of materials due to phytosanitary restrictions and would thus ideally be organized centrally, as previously done by ISI Sementi. Regenerations should be considered as a centralized service for the EVA networks in general that should be funded and could involve professional growers or seed multipliers. An estimate for production of seed from 300 pepper landraces in a small tunnel greenhouse in the Almeria region was given with 20,000 Euros.

Modifications to the current experimental protocol would be the increased number of plants evaluated, review of current traits in evaluation and inclusion of interesting new traits, such as fruit position or evaluation for drought tolerance. The EVA collection should be safety stored (perhaps through a black box arrangement with CGN) and the data used to define a smaller collection reflecting the diversity and most interesting traits for further regeneration and evaluations that could focus more on adaptation. In the Spanish greenhouse production conditions, tolerance for abiotic stresses such as salinity, drought or heat are important traits to investigate further, as they affect both quality and quantity of the produce. Photosynthetic

efficiency and water mobility/availability are important proxies for stress adaptation. Companies could contribute to stress trials with the appropriate protocol.

As next steps for the EVA Pepper network, the genebanks will be invited to join EVA and to explore interest of local breeding companies in their countries in the EVA network. As the EuroPepLand project proceeds with activities in 2025, the EVA Pepper partners will prepare to conduct evaluation trials again in 2026, on EuroPepLand materials and with updated protocols.

The partners agreed to implement specific action points as listed in Annex 3 and the meeting was adjourned after an interesting visit to the greenhouse and post-harvest facilities of Semillas Fito in El Ejido.

# Appendix 1. Meeting agenda

# Agenda

# Venue: Gran Hotel Victoria, Almeria, Spain

DAY 30 OCTOBER			
08:30 - 09:00	Registration		
09.00 – 10:00	Welcome and introductory session	CHAIR: T. CARDI	
09.00 - 09.10	Welcome by local host and ECPGR	<i>M. Fernandez Zurro S. Goritschnig</i>	
09.10 - 09.30	Introduction of participants	All	
09.30 – 10:00	Introduction to ECPGR and overview of the current status of the ECPGR Evaluation Network EVA	S. Goritschnig	
10:00 - 13:00	Results from EVA Pepper project	CHAIR: S. GORITSCHNIG	
10.00 - 10:30	Review of network workplan 2019-2024	S. Goritschnig	
10:30 - 11:00	Preliminary results from additional genotyping	P. Tripodi	
11.00 – 11.30	TEA/COFFEE BREAK		
11.30 – 11:50	Results from analysis of phenotypic data of abiotic trials	G. Batelli	
11:50 – 12:10	Preliminary results from GWAS for abiotic stress	T. Cardi	
12:10 – 13:00	Discussion – further data analysis and dissemination of results	All	
13:00 – 14:00	LUNCH		
14:00 – 17:00	EuroPepLand Intro and Collections	CHAIR: S. GORITSCHNIG	
14:00 – 14:15	Introduction to EuroPepland	P. Tripodi	
14.15 – 15:30	Descriptions of European Pepper landrace collections (~10 minutes per partner)	<ul> <li>M. Bouet INRAE</li> <li>D. Danojević IFVCNS</li> <li>W. v. Dooijeweert CGN</li> <li>A. Antal SCDL</li> <li>R. Giunta CNR-IBBR</li> <li>H. Stavelikova CRI</li> <li>A.R. Burruezo COMAV-UPV</li> <li>S. Ivanovska N. Macedonia</li> </ul>	
15:30 – 16:00	TEA/COFFEE BREAK		
16:00 – 17:00	Descriptions of European Pepper landrace collections (~10 minutes per partner) - online	<ul> <li>U. Lohwasser IPK</li> <li>I. Mellidou ELGO</li> <li>V.B. Masheva IPGR</li> <li>F. Rocha INIAV</li> <li>K. Sarikyan SCVIC</li> </ul>	
20.00	SOCIAL DINNER		

#### Venue: Gran Hotel Victoria, Almeria

DAY 31 OCTOBER	· · · · ·	
9:00 - 10:30	EuroPepLand – work planning	P. Tripodi
	Work planning of EuroPepLand activities - phenotyping - genotyping - data management - data analyis	All
10.30 – 11.00	TEA/COFFEE BREAK	
11:00 - 13:00	EVA Pepper 2.0	T. Cardi
	Integration of EVA Pepper and EuroPepland <ul> <li>Material for field trials</li> <li>Phytosanitary issues</li> <li>Germination issues</li> <li>Regeneration activities</li> <li>Management of EVA and EuroPepland collection</li> <li>timeline</li> </ul>	All
13:00 – 14:00	LUNCH	
	Excursion	
14.00 – 16:30	Visit to Semillas Fito	M. Fernandes Zurro
	Discussion	
16.30 – 18:00	Discussion on continuation of network activities Discussion on workplanning for EuroPepLand Any other open questions	All
18:00	END OF MEETING	

## Appendix 2. List of participants

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# Appendix 3. Action list

#	Activity	Action	Responsible	Due date
1	EVA 2.0	Review EVA membership and invite EuroPepLand genebanks to join	S. Goritschnig	31 Dec 2024
2	EVA 2.0	Genebanks to explore potential interest of breeders in their countries in joining EVA network	S. Goritschnig to coordinate	31 Mar 2025
3	EVA 2.0	Prepare amendment to cooperation agreement to reflect new membership and share with partners for signature	S. Goritschnig	31 May 2025
4	EVA 2.0	Identify regeneration needs for EVA collection and match with capacities within companies.	S. Goritschnig to coordinate	31 Jan 2025
5	EVA 2.0	Update standard protocol with new traits and plant numbers	S. Goritschnig	31 Jan 2025
6	EVA 2.0	Determine available seed stocks and safety duplication needs for EVA collection	S. Goritschnig, T. Cardi	31 Jan 2025
7	Data management	Finalize data curation for missing biotic and abiotic stress trials	S. Goritschnig to coordinate	31 Dec 2024
8	Data management	Provide origin information and available phenotypic data for newly included EVA accessions	P. Tripodi	31 Dec 2024
9	EuroPepLand	Collect input from IPGR Sadovo and INIAV on their collections for EuroPepLand	P. Tripodi	31 Dec 2024
10	EuroPepLand	Provide information for available accessions based on updated shared template	EPL genebanks to P. Tripodi	30 Nov 2024
11	EuroPepLand	Selection of European landraces core collection, highlighting accessions selected for abiotic stress trials	P. Tripodi	31 Dec 2024
12	EuroPepLand	Update EVA standard protocol for phenotyping in EuroPepLand and share with partners	S. Goritschnig	31 Jan 2025
13	EuroPepLand	Develop standard colour scale and ruler for picture taking within EVA networks (also requested by EVA carrot), print, laminate and distribute	S. Goritschnig	31 Jan 2025
14	EuroPepLand	Provide seeds of core collection accessions to CREA-OF for genotyping	EPL genebanks to P. Tripodi	31 Jan 2025
15	EuroPepLand	Regeneration and fruit phenotyping of core collection using standard protocol	EPL genebanks	30 Oct 2025
16	EuroPepLand	Plan inclusion of EPL or EVA accessions in Liveseeding drought trial	A. Rodriguez Burruezo	31 Jan 2025
17	EuroPepLand	Share phenotypic data and regeneration yield of EPL core collection	EPL genebanks, S. Goritschnig to coordinate	30 Nov 2025
18	EuroPepLand	Assign DOIs to all accessions within EPL core collection	S. Goritschnig to coordinate	31 Oct 2025
19	EuroPepLand	Liaise inclusion of EVA and EPL accessions in EURISCO and AEGIS where relevant	S. Goritschnig	31 Oct 2025