

**09 June 2020
09:00 – 12:30, online MS Teams**

Summary report of the meeting

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The meeting substituted for an in-person project meeting on 09-10 June 2020 in Pontecagnano, Italy, and took place on 09 June 2020, 09:30 to 12:30, on MS Teams. The agenda of the meeting is attached as Appendix 1 and the list of participants as Appendix 2.

A number of preparatory documents had been made available to the project partners in advance of the meeting. These included presentations on ECPGR, the EVA networks and EURISCO and its role within EVA, as well as some working documents including a draft experimental protocol, workplan and timeline.

1. Introduction

The EVA coordinator Sandra Goritschnig opened the meeting, reminding participants of the expected outcomes of the meeting and highlighting the available documents. She explained the functions of the virtual meeting platform used and although several participants had problems accessing some of the functions, there were no major problems during the duration of the meeting. In a round of introductions meeting participants indicated their background and expectations for the EVA Pepper network. A number of observers also joined the meeting following an invitation to the ECPGR Solanaceae Working Group.

2. General discussion of the project

2.1 Review of project proposal and current activities in the EVA Pepper Network

The EVA coordinator presented a brief overview of the EVA networks and highlighted the goals of the project as well as ongoing activities and challenges within the EVA Pepper Network. She noted that the networks are still growing and partners are still welcomed to join the various crop-specific networks, informing participants that in collaboration with Euroseeds several additional partners have been identified who expressed interest in joining the EVA Pepper Network. It was confirmed that the project budget for the initial phase of the EVA Pepper Network until 2022 contains funding for genotyping and lab disease assays.

2.2 Update on plant health issues and seed distribution

EVA Pepper Network partners had selected ca. 250 accessions from nine genebanks for multiplication which was planned to happen at ISI Sementi (Italy) as an in-kind contribution, the majority of which was selected from the Centre for Genetic Resources, the Netherlands (CGN). Because of the coming into force of the new European Plant Health Regulation (EPHR) (2016/2031)¹ at the end of December 2019, and emergency measures put into place by the EU to prevent the entry and spread of Tomato brown rugose fruit virus (ToBRFV)², difficulties were encountered in importing seeds from outside the EU. Therefore, 20 accessions provided by the Serbian genebank are currently being multiplied by them.

According to the EPHR 2016-2031, a phytosanitary certificate³ as well as a letter of authority⁴ are required for EU import of pepper seeds for scientific purposes. For distribution within the EU, plant passports⁵ should be issued by competent authorities. The emergency measures in place for

¹ https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/new_eu_rules_en

² https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list_viruses/tomato_brown_rugose_fruit_virus

³ https://ec.europa.eu/food/plant/plant_health_biosecurity/non_eu_trade_en

⁴ <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32019R0829>

⁵ https://ec.europa.eu/food/plant/plant_health_biosecurity/trade_eu_en

ToBRFV⁶ requires that seeds of *Capsicum annuum* to be distributed within the EU or imported into the EU need to be certified (in the plant passport or phytosanitary certificate) to either originate from an area where the pest is known not to occur, or to have been tested for the presence of the virus.

Willem van Dooijewert (CGN, the Netherlands) reported that because of uncertainty in how to apply the new regulations to existing (and often decade-old) genebank material, CGN has decided to no longer distribute seeds for tomato and pepper and are waiting for guidance from the national plant protection organization. Plant passports that declare the seeds free from Potato spindle tuber viroid (PSTV), *Xanthomonas* and ToBRFV are now required for pepper seeds distributed within the EU. PSTV and *Xanthomonas* can be identified by visual inspection, but ToBRFV testing may require sampling of ca. 3,000 seeds, which is problematic for genebank accessions which are usually distributed in much smaller quantities. He noted that it was fortunate that a large number of accessions from CGN had already been included in the EVA pepper project.

He cautioned that material distributed within the EVA Pepper Network would require a plant passport and suggested that multiplying partners should ensure that the plants are inspected by their national plant health service for the three pests before harvest as this could facilitate issuance of certificates and compliance with the EPHR.

Ulrike Lohwasser (Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany) noted that distribution of small quantities for scientific purposes should be possible within the EU without problem; however, she was informed by the German plant health authorities that transfer of Solanaceae material outside the EU is not possible. Teodoro Cardi (Council for Agricultural Research and Analysis of Agricultural Economics (CREA), Italy) questioned whether scientific purposes included experiments in the lab and the field as they have different containment scenarios.

The EVA coordinator reported that they had acquired a letter of authority from the Italian National Plant Protection Organization (NPPO), which allowed import of pepper accessions from North Macedonia now being multiplied by the Italian partner ISI Sementi. She highlighted that this document allows import for scientific purposes and would need to be filed for any material from outside the EU and accompany it throughout the project.

Massimiliano Beretta (ISI Sementi, Italy) commented on the need for testing of multiplied accessions for ToBRFV, suggesting to sample 3,000 seeds from a bulk of accessions for molecular or serological testing. It was noted that the acceptable methods for sampling and testing should be investigated and clarified with the relevant authorities, as well as any necessary paperwork for distribution of seeds across the EU border.

2.3 Update on multiplication activities

M. Beretta presented on the multiplication activities at ISI Sementi. At this moment 233 pepper accessions from 55 origin countries (with 10 countries accounting for 60% of the accessions) are being grown. The majority are cultivated *C. annuum* species, around 40% of accessions are from wild *Capsicum* species. Seed multiplication is done on a minimum of five plants per accession throughout 2020, with harvest expected for the cultivated species by November 2020. Wild species are being monitored for their development in order to ensure effective harvest. Germination data were recorded as well as information on segregation and leaf colour. Sweet and hot peppers have been separated to avoid cross-contamination of pollen. The plan is to produce a minimum of 300 seeds per accession.

⁶ https://eur-lex.europa.eu/eli/dec_impl/2019/1615/oj

Zdenka Girek (Institute for Vegetable Crops, Serbia) provided information on the status of multiplication of Serbian accessions, reiterating the difficulties encountered during export of seeds to the EU. Germination of accessions was around 90% and five plants per accession are being hand-pollinated to ensure purity of the genotypes. Plant health is constantly monitored and harvest is expected in August 2020.

W. van Dooijewert inquired whether it would be possible to organize official inspection of the growing plants to facilitate issuance of phytosanitary certificate and import into the EU, at least to check for PSTV and *Xanthomonas*, as inspection at the end of the multiplication should allow assessment of the plant health.

Teodoro Cardi (CREA, Italy) suggested to establish a Task Force to manage the disease testing. Loredana Sigillo (CREA, Italy) has experience with ToBRFV and could coordinate PCR testing of samples from ISI Sementi. W. van Dooijewert agreed to contribute to the Task Force, M. Beretta and Z. Girek should also be involved and the work will be coordinated by the ECPGR Secretariat.

3. Development of detailed workplan

T. Cardi led the discussion on the workplan, noting that the Network partners should review and agree on a list of traits for evaluation, a suitable trial protocol, and a manageable number of accessions evaluated.

3.1 Traits (laboratory and field)

A list of 66 traits had been assembled including Community Plant Variety Office (CPVO) descriptors, traits evaluated during the Horizon2020 project G2PSol and traits indicated previously by EVA Pepper Network partners. The traits were grouped into morphological traits, traits related to plant structure and agronomic performance, fruit traits, resistance traits tested in the lab and some special or optional traits. He noted that ToBRFV had been included in the list, but since this is now a quarantine pest, tests may need to be reconsidered.

For each trait a proposed scoring scheme was included as well as an indication on whether the trait was interesting and amenable for genome-wide association studies (GWAS). He reminded partners that all accessions would also be genotyped and that therefore the possibility of performing GWAS should be considered in the choice of traits for evaluation. He highlighted that the majority of traits are descriptive and that the final traits list should be agreed based on the capacity of the partners. One possibility presented was to split the accessions and traits based on the pepper species, as GWAS would only be applicable to *C. annuum* accessions which are evaluated at the same time.

One partner asked how to deal with heterogeneous accessions if only a small number of plants are assessed, considering that the majority of the material are landraces and the multiplier has reported on a percentage of segregating accessions. This selection of certain plants within a segregating landrace could lead to missing relevant traits and would also impact the genotyping. It was noted that heterogeneity in accessions would need to be recorded and taken into account. Very heterogeneous accessions may need to be discarded from the evaluation, while maybe for accessions with two haplotypes both could be evaluated. Pasquale Tripodi (CREA, Italy) reported that in the G2PSol project heterogeneous material was often identified as a mix of seeds and suggested that only the most homogeneous accessions be evaluated in EVA, noting that in the multiplication step some heterogeneity would have been purified.

W. van Dooijewert noted that for the CGN material some characterization data and information on heterogeneity was available and could be shared with the partners. He also noted that within the

ECPGR Solanaceae WG a shortlist of ten minimum descriptors for characterization has been developed and that companies consider most important having a picture of the accession.

One partner noted that the traits from the G2PSol are mainly based on IPGRI descriptors and cautioned against using CPVO descriptors, as these are typically based on reference varieties for scaling.

Lorenzo Maggioni (ECPGR, Italy) noted that the list of traits should provide an overview of possible traits for this Network and since mostly breeding companies would provide evaluations in kind, they should select the final trait list based on their interests and capacities. He reminded partners of the main benefit of participating in the EVA Pepper Network, which is the access to evaluation data collected in multiple environments across the greater Europe.

Ifigeneia Mellidou (Institute of Plant Breeding and Genetic Resources – Hellenic Agricultural Organisation (HAO)-Demeter, Greece) noted that the main interest should focus on traits related to biotic and abiotic stresses or nutritional values and that the CPVO descriptors are more useful for characterization by genebanks.

Robert Legnani (Takii, France) noted that for Takii it would be better to phenotype more accessions on fewer traits. M. Beretta suggested that traits for which molecular markers are already available could be excluded from the traits list. W. van Dooijewert noted that the CGN materials have been characterized in greenhouse environment, so perhaps field evaluations could be used to confirm and complement existing data. He also suggested that traits with linkage to molecular markers would be most interesting to phenotype.

T. Cardi noted that the wild accessions included in the material would be interesting for different traits than the cultivated *C. annuum* and therefore suggested that the available accessions could be split accordingly.

T. Cardi briefly introduced the draft trial protocol and invited partners to review it, noting that the specifics of experimental design and crop management would depend on the trial capacity of partners and the finalized traits list.

L. Sigillo provided some information on the design of the laboratory disease tests planned for Tomato spotted wilt virus (TSWV) and *Verticillium*, noting that the selection of pathogens had been done during the EVA Vegetables meeting in Durres, Albania in 2019⁷ and could be revised based on preferences of partners. She also noted that because ToBRFV has been assigned as a quarantine pest in Italy these assays can no longer be done by CREA and other partners were invited to offer to do these tests if possible.

One partner noted that likely ToBRFV might be the most interesting resistance trait for companies, given that it is an emerging pest in Europe. Another partner noted that they did not consider *Verticillium* a priority pest and asked why it had been included in the original trait list. L. Sigillo reported on the original considerations during the meeting in Durres, which were based on bibliography and the proposal to test a viral and a fungal pathogen, and *Verticillium* was selected as a seed borne pathogen. In addition, a recent emergence of *Verticillium* has been reported in Southern Italy, suggesting changes in its virulence, which may make it an interesting pathogen to screen. She added that *Phytophthora* could be another interesting pathogen to test, however, maintenance is difficult and the choice of strain would also need to be discussed, noting that within the European specialist network working with CPVO no suitable strain has been identified. Other pathogens could be proposed by partners and included in the list if agreed.

⁷ Workshop for the establishment of a European Evaluation Network (EVA) on vegetables. Durres, Albania, 2-3 April 2019.

In response to a partner's question on which TSWV strain would be used in tests, L. Sigillo confirmed that they would use a resistance breaking race 1 strain for which no resistance is known among commercial varieties, adding that the purity of the inoculum would be verified, most likely by using a differential set of accessions. T. Cardi added that no resistant varieties are known to the TSWV strain used in the tests, and the intention of the screen was to identify new sources of resistance among the genebank materials. He suggested that disease tests could include those for which no reliable molecular markers are currently available.

It was agreed to provide a survey so that evaluators can score their preference for the traits and from which a shortlist of 10 to 20 traits can be developed. The list would be informed by the current proposal and discussions during the meeting, as well as the minimum descriptors and available characterization data for CGN accessions. The survey would also assess under which conditions trials would be held at different evaluation partners and how many accessions each partner is capable to evaluate, keeping in mind that for a GWAS analysis around 150 accessions would need to be evaluated at the same time.

3.3 Genotyping

T. Cardi introduced the proposed genotyping plans as defined during for the EVA pepper project proposal, which included genotyping all accessions (ca. 200) with genome-wide markers (commercial provider, coordinated by P. Tripodi, CREA) and with trait-associated markers, focusing mainly on disease resistance (Institute of Genetics and Cytology (IGC), Belarus) in 2021.

Olga Babak (IGC, Belarus) informed that the institute is ready to implement the first part of the genotyping strategy according to the trial protocol, performing the analysis using molecular markers associated with disease resistance genes. She agreed with using markers and protocols from Di Dato et al. (2015)⁸, noted that additional markers published later could also be included and shared a table with possible additional markers. She noted the need to discuss how material for genotyping would be shared with them, considering the difficulty in transferring seeds.

P. Tripodi noted he would oversee the whole genome single-nucleotide polymorphism (SNP) genotyping, which was proposed to use the SNP chip array developed by the Pepper Consortium⁹. This array has been developed based on sequencing data of 22 pepper lines, which after filtering for quality parameters led to 16 405 SNPs. It was indicated that an improved array may be available in the near future from other consortiums and that the most updated version will be checked at the time of genotyping.

He noted that arrays are usually supplied as 96-well plates and therefore a multiple of 96 accessions should be genotyped to make best use of available space. Quotations would be asked from several commercial providers for generation of raw data as well as some pre-analysis (e.g. SNP calling) and current cost estimates would be around 4000-5000 € per plate.

The DNA for the SNP chip arrays will need to be of a high quality, therefore P. Tripodi would be coordinating the DNA extraction at CREA as well as the shipping of DNA to commercial providers. He noted that ideally the same DNA should be used for marker analysis at IGC and that small aliquots could be shared with the partners in Minsk.

3.4 Workplan

The EVA coordinator introduced shared documents to facilitate coordination of project activities, including a time plan, a survey of evaluation sites and roles and responsibilities of partners. T. Cardi added that among the activities for partners it will be important to identify those interested

⁸ <https://link.springer.com/article/10.1007/s10681-014-1345-4>

⁹ <https://www.nature.com/articles/hortres201636#Sec13>

in contributing to a joint analysis of the generated phenotypic data, noting that CREA could contribute to GWAS analysis. Methods of data analysis will depend on the traits evaluated and expertise among partners and could be discussed during a future meeting.

Stephan Weise (EURISCO coordinator) clarified that work is ongoing on generating common data exchange templates, which will facilitate data management and that an EVA network intranet, set up as an extension to EURISCO, will allow partners to access all data generated from all evaluations within the network. In response to a partner's question on how the data will be integrated in the public EURISCO environment after the embargo, he noted that this is still under discussion and that eventually an online upload tool would be developed for use by the EVA networks.

Participants were invited to indicate what their capacity for evaluations would be and in which environments they would be able to do evaluations.

Company partners noted that they would be able to evaluate in open field, others could evaluate both in open field and greenhouse, with greenhouse space more limited, noting that the final capacity for evaluation would depend on the traits selected and the associated workload.

I. Mellidou noted that HAO-Demeter would only be able to evaluate a subset of accessions in 2022. M. Beretta indicated that a contribution of ISI Sementi to evaluation would need to be discussed with management, considering that the in-kind multiplication of pepper accession already required a lot of resources.

Sonja Ivanovska (Ss. Cyril and Methodius University in Skopje, North Macedonia) noted that their capacity is limited and their main interests would be on their own local varieties. L. Maggioni suggested that this material could be included in a possible second round of evaluations provided that sufficient seeds are available and S. Ivanovska confirmed that they had enough seed of ca. 250 accessions of embroidered peppers as well as an additional ca. 700 accessions of peppers collected from local farmers, which could be shared.

The EVA coordinator welcomed this proposal and reminded participants that the intention of the EVA Pepper Network was to evolve into a self-sustaining network that continues beyond the current project duration to generate evaluation data from material available in European genebanks. She noted that if partners were in favour of continuing the network activities in a rolling circle, a new set of accessions should be identified for multiplication possibly in 2021.

4. Next steps

Based on the discussions during the virtual meeting and input from the project partners, the following next steps were agreed:

1. In order to comply with EU plant health regulations of multiplied material, a Task Force of network partners (W. van Dooijewert, L. Sigillo, M. Baretta, Z. Girek and S. Goritschnig) will investigate acceptable methods for sampling and testing for ToBRFV and clarify with the relevant authorities, as well as any necessary paperwork for distribution of seeds across the EU border.
2. The multipliers (M. Beretta and Z. Girek) will arrange for inspection visits by competent authorities before harvest to facilitate issuance of plant passports.
3. W. van Dooijewert will share with partners existing characterization data for accessions provided by CGN.
4. Quotations for genotyping will be gathered from commercial providers and the relevant activities and methods defined.

5. A questionnaire will be developed by L. Sigillo and S. Goritschnig to survey partners' preferences for traits of interest as well as their evaluation environments and capacity. Other possible items included in the questionnaire could be trait-specific markers of interest, a survey of capacity for disease resistance assays and expertise for data analysis as well as on the partners' interest in continuing with a second round of multiplications and evaluations on new material.
6. Results of the survey will be made available to partners and discussed during a future meeting, which will hopefully be held in person and in conjunction with a meeting of the ECGPR Solanaceae WG.

In a post-meeting survey participants expressed general satisfaction with the outcomes of the meeting, although it was clear that additional discussions will be required to clarify existing issues as outlined above and to finalize a work plan of the EVA Pepper network. Of particular importance will be to attract additional partners to the network to expand the geographic range of evaluation sites.

Appendix 1. Meeting agenda

PRE-MEETING DOCUMENTS

ppt	Background and overview of the ECPGR Evaluation Network EVA	L. Maggioni
ppt	Update on current status and activities in EVA Network - Focus on pepper component	S. Goritschnig
ppt	EURISCO: ensuring integration of data in special intranet environment for EVA	S. Weise
Documents	Drafts available for: <ul style="list-style-type: none"> • EVA Pepper project proposal • Project workplan and timeline • Roles and responsibilities of partners • List of traits for selection • Cooperation Agreement 	S. Goritschnig

09 June, 9:00 – 12:30 (Venue: MS Teams)

9:00 – 9:30	Connecting to MS Teams – technical assistance if needed	
	Welcome	
9:30 – 9:35	Welcome and introduction of platform and available files/tools	S. Goritschnig
9:35 – 9:45	Introduction of participants	All
	General discussion of the project	Chair: S. Goritschnig
9:45 – 10:15	Review of project proposal and current activities in the EVA network Pepper Update on plant health issues and seed distribution Update on multiplication activities	S. Goritschnig S. Goritschnig/ W. van Dooijewert M. Beretta / Z. Girek
10:15 – 10:30	Q&A	
10:30 – 10:45	Break	

	Development of detailed work plan	Chair: T. Cardi
10:45 – 12:00	<p>Discussion to reach agreement on:</p> <p><u>Traits (Laboratory and field)</u></p> <ul style="list-style-type: none"> • selection of traits of interest • descriptors for traits to be evaluated • standard experimental protocols <p><u>Genotyping</u></p> <ul style="list-style-type: none"> • Review of protocols and data analysis pipeline <p><u>Workplan</u></p> <ul style="list-style-type: none"> • roles and responsibilities of each project partner • confirmation of evaluation sites • timeline of project activities <p><u>Second round of evaluations</u></p> <ul style="list-style-type: none"> • need and volunteers for multiplication of accessions • selection of accessions 	All
12:00 – 12:30	<p>Wrap-up of meeting: Review of cooperation agreement, timelines and deliverables Define next steps</p>	S. Goritschnig

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Appendix 3. Acronyms and abbreviations

CGN	Centre for Genetic Resources, the Netherlands
CPVO	Community Plant Variety Office
CREA	Council for Agricultural Research and Analysis of Agricultural Economics, Italy
DNA	Deoxynucleic acid
ECPGR	European Cooperative Programme for Plant Genetic Resources
EPHR	European Plant Health Regulation
EU	European Union
EURISCO	European Internet Search Catalogue
EVA	European Evaluation Network
GWAS	genome-wide association studies
HAO	Hellenic Agricultural Organization, Greece
IGC	Institute of Genetics and Cytology, Belarus
IPGRI	International Plant Genetic Resources Institute
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
NPPO	National Plant Protection Organization
PCR	polymerase chain reaction
PSTV	Potato spindle tuber viroid
SNP	single-nucleotide polymorphism
ToBRFV	Tomato brown rugose fruit virus
TSWV	Tomato spotted wilt virus