Annual meeting of the
European Evaluation Network (EVA) for Pepper

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

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The 2021 annual meeting of the EVA Pepper network took place on 14 June 2021, 09:00 to 12:00, on MS Teams. 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 virtual meeting, welcoming project partners and observers from the ECPGR Solanaceae Working Group. She reminded participants of the expected outcomes of the meeting and highlighted available documents in the network's sharepoint folder. She expressed the hope and intention of holding the next annual meeting in person to facilitate strategic discussions and noted that this will be planned back-to-back with a meeting of the Solanaceae WG and hosted by CREA Pontecagnano.

2. Review of project and general update

2.1 Update on EVA

Sandra Goritschnig updated partners on several developments within the network, informing them of a no-cost project extension until November 2023 which had been granted by the German donor. This extension will allow the individual networks to finalize activities that were disrupted or delayed by the Covid-19 pandemic and also provide the opportunity to hold in-person meetings, important for strategic discussions, about possible continuation of the projects. Partners were reminded to return signed cooperation agreements to the ECPGR Secretariat by 30 June 2021 to finalize this administrative aspect of the project. The EVA-EURISCO intranet is under construction and the EVA webpage had been updated; partners were invited to provide feedback on both when necessary.

Given that phytosanitary issues have proven important obstacles for the EVA Pepper network, partners were informed of an online workshop on "Phytosanitary issues for genetic resources", which had been organized by the ECPGR Secretariat within the framework of the H2020 project GenRes Bridge. Preparatory webinars and presentations given during the workshop, as well as a report, are available online[1]. Willem van Dooijeweert presented the difficulties experienced by CGN in dealing with the new EU Plant Health Regulation (2016/2031)[2], with reference also to the issue with ToBRFV on access to their tomato and pepper collections. W. van Dooijeweert informed partners that CGN, together with other national gene banks, had sent a message to the SCoPAFF (Standing Committee on Plants, Animals, Food and Feed) of the European Commission to request an exemption from testing requirements for gene bank material in storage since before the emergence of the relevant pest, as it should now be considered free of the pest.

2.2 Review of project workplan and 2020 activities

Multiplication activities had been split between partners ISI Sementi in Fidenza, Italy and the Institute for Vegetable Crops (IVC) in Smederevska Palanka, Serbia. Both partners organized phytosanitary inspections during the growing season and also ToBRFV PCR tests on harvested seeds to ensure the phytosanitary health of the multiplied material. For the ToBRFV tests 29 seeds from each accession were pooled in reactions of up to 3000 seeds, in accordance with the annex

[1] https://www.ecpgr.cgiar.org/working-groups/common-working-group-activities/phytosanitary-workshop
[2] http://data.europa.eu/eli/reg/2016/2031/oj

of EU regulation (2019/1191) on ToBRFV testing[3] and the International Standard on Phytosanitary Measures (ISPM) 31 on methodologies for sampling of consignments[4]. Thus, the material was certified free of any regulated pests relevant to the European Union and import of seeds from Serbia to Italy proceeded without problems.

The seeds have been distributed by ISI Sementi to all partners within the EU. At the moment, however, the seed shipment from Italy to the partner in Armenia was delayed because of missing documentation. Partners were reminded of the importance of effective communication, especially relevant for phytosanitary regulations, to ensure timely processing and delivery. Yonatan Elkind (Hebrew University) noted that while Israel did not have problems with ToBRFV (because it's not regulated), he had finally established what documentation is necessary for import of pepper seeds for his experiments.

Based on the network's experience with ToBRFV testing, it may be possible to access additional gene bank material for future evaluation cycles, especially if gene banks themselves could do the multiplications. W. van Doojeweert reported that CGN had started distributing newly generated genetic material after testing using the sampling and pooling scheme described above. He cautioned, however, that the required sample size increased with the size of the tested seed lot and may therefore reduce the number of accessions that could be tested in one pooled reaction.

The multiplications of 160 accessions yielded sufficient seed for all scheduled field and lab trials and for genotyping, 140 provided by ISI Sementi and 20 by IVC. An additional 22 accessions had enough seeds for lab trials and genotyping, but inclusion in field trials would require a secondary multiplication. Pasquale Tripodi (CREA) noted that he could use some of the plants sampled for genotyping to regenerate more seeds in the greenhouse. Yonatan Elkind commented that in some cases, it may be useful to stress plants in order to induce fruit set.

Eight network partners were conducting field trials on the pepper accessions, three of which have limited capacity and will jointly provide two datapoints per accession. Two partners are conducting lab trials on important diseases. Together, the network thus evaluates the EVA pepper collection across 10 evaluation sites/environments.

3. Preview of activities 2021/2022

Several partners had already started with their field experiments and reported that for some accessions, they had noticed low germination. Partners were asked to provide detailed information about the identity of accessions with low germination and this will be compared with seed yields from multiplications to make seeds available for trials in 2022, where possible.

3.1 Review of workplan and experimental protocol.

Teodoro Cardi led the discussions in this section and presented the experimental plan as discussed in the previous meeting, where partners had agreed on using a block design including two replicates for each accession. However, based on feedback received from partners it seems that most who have already planted their trials only included one replicate. This should nevertheless be sufficient to generate good quality data on the selected traits, which are considered relatively stable.

[3] http://data.europa.eu/eli/reg_impl/2020/1191/2021-01-30
[4] https://www.ippc.int/en/publications/588/

Partners individually commented on the progress of their field trials, noting also accessions with low germination rates. In general, depending on germination, between one and eight plants per accession were available, and these will be evaluated according to the standard protocol.

Massimiliano Ballardini (Esasem) informed that their trial would be in open field but they have also included two plants per accession in the glasshouse, providing an additional environment for evaluation. Zdenka Girek (IVC Serbia) noted that due to bad weather conditions, their transplanting was somewhat delayed but they planned to also use plants in the greenhouse for seed increase. Partners Semiorto (Italy) and Scientific Centre of Vegetable and Industrial Crops (Armenia) were not present at the meeting and would be contacted individually to provide their feedback. All feedback had been collected in a shared file available to network partners and should be updated as necessary.

Teodoro Cardi reminded partners of the traits in evaluation and the agreed number of plants that should be scored for each accession, outlined in the experimental protocol. He suggested that for structural traits, if only one repetition is planned per experiment, more plants should be evaluated where possible to ensure sufficient data for statistical analysis.

He highlighted the need to note homogeneity of the accessions, preferably selecting similar plants for the scoring. W. van Dooijeweert noted that since most accessions were landraces, they were not necessarily uniform and differences within accessions should be considered in the evaluations. When multiplying gene bank accessions at CGN, the seeds are collected heterogeneously, combining seeds from multiple plants and fruits, to specifically maintain the diversity within the landrace. This is despite the fact that the seeds in evaluations have been multiplied from single plants. In addition, it was pointed out that sometimes, even in genetically uniform pepper material, there can be quite some variation, perhaps due to environments. Partners were asked to collect data from similar plants with the most prevalent phenotype but also to take note of any heterogeneity observed within accessions.

3.2 Review of standard protocol for field trials

Sandra Goritschnig guided the participants through the standard protocol, which had been compiled to provide guidance on scoring traits evaluated during field and greenhouse trials and was based on the FAO/IPGRI Descriptors for pepper[5] and the CPVO technical protocol for Capsicum annuum (v.2.2_0)[6]. It was complementary to the data collection template and included pictorial guidance where available.

The partners reviewed the content and commented on specific traits:

Capsaicin content – the IPGRI trait was scored as absent and present, it was suggested to consider including intermediate levels. However, for quantifying intermediate scores biochemical analyses may be necessary. In order to make scoring as simple as possible, it was agreed to score only presence/absence and to perhaps take note of any exceptionally pungent samples. Ifigeneia Mellidou (HAO Demeter, Greece) informed that summer students would be working on the project, scoring the material for pungency (pun markers). Another comment raised the diversity of the materials, questioning how many fruits should be sampled per accession, noting also that pungency could be detected by smell. It was also noted that the pun markers were included in the set for marker genotyping done by IGC Minsk.

Growth habit: it was noted that the growth habit could present differently dependent on the environments the plants were grown in (field vs greenhouse vs tunnel). In the field, the plants would display a more natural habit, and this is where it should be scored, while in commercial

[5] https://www.bioversityinternational.org/e-library/publications/detail/descriptors-for-capsicum-capsicum-spp/
[6] https://cpvo.europa.eu/sites/default/files/documents/capsicum_annuum_2.2_0.pdf

growing facilities this would be adjusted through pruning. It was highlighted that evaluators should note any treatments and their experimental setup in the data collection template.

Time of maturity: the CPVO trait is scored on a scale of early to late. However, since the relevant controls were not included in the trial, it was suggested to adjust to days after transplanting. A relative score could then be generated for the different experiments based on all trial data, which would then allow easier comparison of the data. Partners were reminded that is was important to include the observation date for any observed trait score and that it was important to score traits using the same methods in order to allow comparison.

3.3 Preview of genotyping activities

Pasquale Tripodi (CREA) provided an overview of the planned activities. The SNP genotyping would be done with a high density array developed by a UC Davis/Trait Genetics consortium (https://doi.org/10.1038/hortres.2016.36), available from Illumina. The advantage of this approach was that results don't require complicated bioinformatics analyses and the use of standard SNPs allows easy comparison with previous and future experimental datasets. The analyses that are planned for the dataset were, where possible, marker analysis (PIC, heterozygosity, etc.), population genomic inferences (structure, phylogenesys, PCA, similarities, etc) and genome wide association mapping.

182 accessions were planned to be included in the genotyping, and leaf material from up to five plants would be sampled, pooled and processed by CREA and distributed to the third party genotyping provider. In addition, lyophilized tissue would be shared with partner IGC Minsk (Belarus), who would be genotyping with gene-specific markers linked mostly related to disease resistance and pungency. It should be noted that not all markers were directly on associated resistance genes. The L4 resistance gene has been shown to confer resistance to ToBRFV, but not all L4 markers described in the literature show good correlation. Primer sequences of these primers were shared with partners.

Genotyping results can be expected by autumn 2021 and initial analyses as outlined will be performed. Additional analyses could be done if there was interest and some relevant research questions. It was noted that using bulked material for genotyping may complicate genome wide association studies, but could provide useful information on heterozygosity of the material. Which analyses were possible would depend on the quality and variability of the phenotypic data generated in the different locations and would be discussed later on, also considering that the phenotyping of all accessions would be done over two years.

3.4 Preview of lab disease tests

Loredana Sigillo (CREA) provided an overview of the planned laboratory trials for Tomato Spotted Wilt Virus (TWSV) and Xanthomonas euvesicatoria, which would be performed in 2021 and 2022, respectively. In preparation for the TSWV tests, suitable resistance-breaking strains were obtained from the Italian National Research Council and a standard protocol based on the UPOV protocol for TWSV (TG/76/8 rev 2[7]) was being tested and optimized. Initial results on the project material can be expected to become available by autumn 2021. The scoring scale for the resistance scoring may be extended from absence/presence to include intermediate values if necessary. Reinoculations of material would ensure that results were homogeneous and it should also be noted that repeated multiplications may decrease the virulence of TSWV, therefore the inoculation would be done on all material at the same time. Replications would be necessary to confirm suitable resistant candidate accessions.

[7] https://www.upov.int/edocs/tgdocs/en/tg076.pdf

Similarly, a selection of an optimal strain of X. euvesicatoria and development of a screening protocol were under way, and tests on the project material would be performed in 2022.

Yonatan Elkind reported on the requirements for import of pepper seeds to Israel which were necessary for a permit so that they could be imported without further testing. The experiments for ToBRFV resistance would then be performed in quarantine facilities and could start in August/September 2021.

3.5 Review of data collection template

Sandra Goritschnig presented the data collection template which should be used by partners to record the field trials. A guidance document for the template was available and other networks were using the same standardized template, which should facilitate upload of phenotypic data into the EVA-EURISCO data repository.

The data collection template was an excel file with several worksheets providing information on the tested accessions, experimental setup and metadata, trait descriptions including scoring scales and allowed values, and a worksheet where observed data would be submitted and linked to the relevant metadata.

Partners were invited to review the template and identify their trial, providing relevant metadata. Based on discussions during the meeting, some trait information would be updated and a finalized document shared with partners in due course. Data collection templates for lab trials would be developed separately.

4. Next steps

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

1. Protocols, data templates and experimental plans would be updated to reflect decisions made during the meeting
2. Partners who were not present at the meeting would be asked to provide feedback on their actual field trials setup
3. Paperwork for exporting pepper seeds to Israel would be coordinated between Hebrew University, ISI Sementi and IVC Serbia.
4. Partners would provide information on germination in their trials and, where necessary, and available, additional seed would be provided for trial replicates in 2022
5. Missing signatures for the cooperation agreement would be collected and a compiled document prepared as soon as possible.
6. CREA (Pasquale Tripodi) would grow accessions that did not yield sufficient seed during the first multiplication to potentially provide seeds to partners for 2022
7. Quotations for genotyping would be obtained from third party providers by Pasquale Tripodi and Sandra Goritschnig
8. Sub-agreements for the lab and genotyping activities would be finalized with relevant partners.

A future in-person meeting was still planned to happen in conjunction with a meeting of the ECPGR Solanaceae Working Group, but this would likely only be possible next year. A virtual meeting to discuss first results from all trials would be scheduled in December 2021, after the end of this year's field trials.

In a post-meeting survey, participants expressed general satisfaction with the progress of the project. Some partners noted Covid-19 related delays or difficulties in their activities but expressed confidence in being able to provide high-quality evaluation data in their trials.

Appendix 1. Meeting agenda

14 June 2021, 9:00 – 12:00 (Venue: MS Teams)

08:45 – 09:00	Meeting room opened; technical assistance if needed	
	Welcome	
09:00 – 09:05	Welcome and review of platform and available files/tools	S. Goritschnig
	Review of project and general update	**Chair: S. Goritschnig**
09:05 – 09:15	Review of project proposal and general update Phytosanitary issues Multiplication activities 2020	S. Goritschnigber
	Preview of activities 2021/2022	**Chair: T. Cardi**
09:15 – 09:30	Review of work plan and experimental protocol	All
09:30 – 09:45	Review of standard protocol for field trials	All
09:45 – 10:00	Preview of genotyping activities	P. Tripodi
10:00 – 10:15	Preview of lab disease tests	Y. Elkind L. Sigillo
10:15 – 10:30	**Break**	
	Outlook	**Chair: T. Cardi**
10:30 – 11:00	Review of data collection template	S. Goritschnig
11:00 – 11:45	General discussion	All
11:45 – 12:00	Any other business	All
12:00	Close of meeting: Next meeting (as necessary): date tbd	S. Goritschnig

Appendix 2. List of participants

Massimiliano Ballardini
ESASEM SpA
Via G. Marconi 56
37052 Casaleone (Verona)
Italy
Email: mballardini@esasem.com

Enrico Belfanti
Terra Seeds srl
Via della Rotaia, 4
47035 Gambettola
Forli Cesana
Italy
Email: ebelfanti@teraseeds.com

Teodoro Cardi
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria,
Centro di ricerca Orticoltura e Florovivaismo
Via Cavalleggeri, 25
84098 Pontecagnano Faiano (SA)
Italy
Email: teodoro.cardi@crea.gov.it

Willem van Dooijeweert
Centre for Genetic Resources, the Netherlands (CGN)
PO Box 16
6700AA Wageningen
Netherlands
Email: willem.vandooijeweert@wur.nl

Yonatan Elkind
The Hebrew University
Sherman Building
Edmond J. Safra Campus
9190401 Jerusalem
Israel
Email: Yonatan.elkind@mail.huji.ac.il

Marta Fernández Zurro
Semillas Fitó S.A
Selva de Mar 111
Barcelona 08019
Spain
Email: mfernandezz@semillasfito.com

Zdenka Girek
Institute for Vegetable Crops
Karadjordjeva 71
11420 Smederevska Palanka
Serbia
Email: zdnkgirek@yahoo.com

Ulrike Lohwasser
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
Corrensstr. 3
06466 Seeland OT Gatersleben
Germany
Email: lohwasse@ipk-gatersleben.de

Ifigeneia Mellidou
Hellenic Agricultural Organization (HAO) – DEMETER
Institute of Plant Breeding and Genetic Resources
57001 Thermi
Thessaloniki
Greece
Email: ifimellidou@gmail.com

Chiara Milanesi
Consorzio Sativa
Via Calcinaro 2425
47521 Cesena (FC)
Italy
Email: cmilanesi@sativa.it

Loredana Sigillo
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agrarian (CREA),
Centro di ricerca Orticoltura e Florovivaismo
Via Cavalleggeri, 25
84098 Pontecagnano Faiano (SA)
Italy
Email: loredana.sigillo@crea.gov.it

Rebecca Stevens
INRAE, Génétique et Amélioration des Fruits et Légumes
UR 1052 Domaine St Maurice,
Allée des Chênes
CS 60094
84143 Montfavet cedex
France
Email: Rebecca.stevens@inrae.fr

Pasquale Tripodi
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agrarian (CREA),
Centro di ricerca Orticoltura e Florovivaismo
Via Cavalleggeri, 25
84098 Pontecagnano Faiano (SA)
Italy
Email: pasquale.tripodi@crea.gov.it

ECPGR Secretariat

Sandra Goritschnig
The Alliance of Bioversity International/CIAT
Via di S. Domenico 1
00153 Rome
Italy
Email: s.goritschnig@cgiar.org

Vanessa Bryant
The Alliance of Bioversity International/CIAT
Via di S. Domenico 1
00153 Rome
Italy
Email: v.bryant@cgiar.org