

**Summary report of the**  
**Annual meeting of the**  
**European Evaluation Network (EVA) for Pepper**  
**26–28 October 2022**  
**Vico Equense, Italy**



Illustrations: Loredana Sigillo

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The 2022 annual meeting of the EVA Pepper network took place from 26-28 October 2022 in Vico Equense, Italy. Due to some last-minute cancellations, several project partners could not attend in person, therefore the meeting was held in a hybrid format. 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. Teodoro Cardì, local coordinator and former director of CREA Pontecagnano, welcomed meeting participants and expressed the hope that despite the unfortunate cancellations which reduced the number of partners attending in person, the meeting would allow useful discussions and provide sufficient guidance for the network's work going forward.

After a round of introductions, S. Goritschnig noted that the planned visit to CREA Pontecagnano had been cancelled and the agenda of the meeting adjusted accordingly.

## **2. Results from the EVA Pepper project**

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

S. Goritschnig updated participants on developments within the EVA Pepper and other networks. The EVA Pepper network currently has 15 partners from 10 countries, including 6 breeding companies. ISI Sementi, which has recently been acquired by the Mitsui group, is currently restructuring and divesting its pepper breeding project, and will therefore no longer be able to contribute to the EVA Pepper network. Negotiations are ongoing to identify a substitute company from within the Mitsui group, who would take over the role of ISI Sementi.

So far, three EVA networks have held in-person project meetings in 2022, and one more is planned for EVA Wheat and Barley in November. In general, project partners appreciated the opportunity to interact in person, which enabled effective discussions on the remaining work to be done during the current project, focusing mainly on data analysis, and also facilitated a dialogue between partners on future collaborations in extensions of the EVA networks. The EVA project has been promoted on multiple occasions, by the EVA coordinator as well as EVA carrot network partners who presented their work during two international congresses in 2022. This project outreach has resulted in discussions which have started among other ECPGR crop Working Groups who are interested in initiating new EVA networks.

S. Goritschnig updated on recent developments in relation to Tomato Brown Rugose Fruit Virus (ToBRFV). The EU emergency measures in place for this pest are due to expire in May 2023. Over the past years, the virus has been detected in most European countries, making it difficult to be managed as a quarantine pest. Therefore, several National Plant Protection Organizations (NPPOs) from Italy, Greece, Spain, the Netherlands, as well as the industry sector, are proposing to change the pest status of ToBRFV to that of a "Regulated Non-quarantine Pest" (RNQP), which would allow the focus to shift to mitigation instead of eradication. A decision by the Standing Committee on Plants, Animals, Food and Feed (SCOPAFF) on this is expected in March 2023.

## 2.2 Review of network workplan 2019–2023

S. Goritschnig provided an overview and summary of the work plan agreed for the EVA Pepper network. Activities included the multiplication of more than 200 pepper accessions in 2020, field, greenhouse and lab evaluations as well as genotyping in 2021 and 2022, with a plan to focus work on the analyses of the generated datasets in 2023.

Eight network partners are 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. Karine Sarikyan (Scientific Centre of Vegetable and Industrial Crops, Armenia) noted that because of delays in customs they did not receive seeds in time for trials in 2021 but had collected data in 2022 and were planning on conducting another trial in 2023.

So far only four datasets had been successfully uploaded to the EURISCO-EVA intranet, and partners were reminded to provide their data for curation as soon as possible. Some open questions with regard to data curation were highlighted for discussion during the meeting.

## 2.3 Review of selected accessions and regeneration activities

Partners were reminded of the process and criteria employed in the selection of the plant material evaluated in the project.

Willem van Dooijeweert (CGN, the Netherlands), having provided the majority of pepper accessions from their collection, gave an overview of the current CGN pepper collection and an outlook on acquisitions which may enter the catalogue in the coming years. The CGN collection currently contains 1,177 *Capsicum* accessions, the majority of which are *C. annuum*. Some evaluation data for resistance traits are available on EURISCO for more than 950 accessions, an important factor in selecting material for EVA Pepper. Molecular data have been produced for ~800 accessions within the G2PSol project and the Capsicum Genome Initiative, and data will be made available on the CGN website after an embargo period. He explained the procedures used by CGN in regeneration, maintenance, storage and distribution of pepper genetic resources and highlighted that additional material could be made available for a future round of EVA pepper. Pepper regeneration is typically done in collaboration with breeding companies who contribute in kind. Seven plants are used for homogeneous accessions and 14 for heterogeneous landraces, and seeds from the same amount of fruits harvested from each plant are pooled after cleaning. Seeds are stored under low oxygen conditions at -20°C for up to 50 years with first germination tests conducted after 25 years.

Zdenka Girek (Institute for Vegetable Crops, Serbia) presented her institute's small collection of ~100 pepper accessions. The pepper collection in Smederevska Palanka is regenerated every three years and maintained as a working collection. Twenty accessions were contributed to the EVA project. These had been previously characterized and were considered useful for breeding. Due to the emergency measures introduced for ToBRFV at the end of 2019, the seeds could not be sent to multiplying partner ISI Sementi and were therefore multiplied in Serbia. After phytosanitary testing, seeds were distributed to evaluation partners and in 2021 all accessions were remultiplied for a second round of evaluations.

Pasquale Tripodi (CREA, Italy) recalled the strategy employed in selecting accessions for the network. Firstly, participating companies were asked for their input and confirmed a primary interest in resistant materials. Secondly, the selection aimed at maximizing the diversity of the collection based on existing characterization data from CGN and G2PSol. Thirdly, with a view

towards future use in breeding, the collection focused on *Capsicum* species that could be crossed with *C. annuum*. Genotyping of the collection has confirmed that the selected EVA pepper collection is highly diverse.

## 2.4 Preliminary results of pepper genotyping

P. Tripodi (CREA) presented preliminary results from genotyping of 161 pepper accessions with a high density 19k SNP array developed by a UC Davis/Trait Genetics consortium (<https://doi.org/10.1038/hortres.2016.36>), available from Illumina. After filtering and some quality control steps, around 14k SNPs constitute the final dataset. These are mostly associated with genic regions covering the whole pepper genome. A high heterozygosity level (above 40%) was observed for a number of accessions, which should be correlated with phenotypic data. Admixture analyses indicated that the molecular data well correlates with the taxonomy of the collection. However, some accessions may have been misclassified and this information will be shared with holding genebanks in order to revise passport data as necessary. Neighbour-joining analysis also clearly separates *C. annuum* and wild species.

Although the number of accessions is on the low side, genome-wide association studies (GWAS) may be possible on the *C. annuum* accessions, provided the phenotypic data are robust across locations. Several models can be used to conduct GWAS, with different parameters. GWAS could be done on traits for which the collection covers the whole range of possible trait scores.

## 2.5 Preliminary results from lab trials

Yonatan Elkind (Hebrew University, Israel) communicated preliminary results from lab experiments with ToBRFV but could not present them himself. In a first replicate of the experiment, more than 50% of accessions had not germinated well and many accessions had fewer than the desired ten plants per experiment. Nevertheless, several accessions of *C. annuum* and *C. chinense* were identified for which resistance could be confirmed by ELISA and reinfection of *Nicotiana glutinosa*. The experiments will be repeated with all accessions to allow testing also of those that had previously not germinated.

Eliana dell'Olmo (CREA) presented results for the lab trials on *Xanthomonas euvesicatoria* on behalf of Loredana Sigillo (CREA). Resistance responses were assessed using a 4-level scoring scale, where 1 signifies resistant and 4 heavily diseased. *C. chinense* accessions used a different scale as no symptoms of level 4 were observed. Most of the tested samples were susceptible but several resistant and intermediate-resistant accessions were identified among *C. annuum* and *C. chinense*.

## 2.6 Preliminary results from field trials

Project partners presented summary results and feedback from their trials conducted in 2022.

K. Sarikyan reported on the trial conducted between April and October 2022 in their field station located in Darakert, Armenia at ~840masl. The Scientific Centre has been working on solanaceous crops for more than 70 years. All accessions had been sown but only 90 had germinated well enough to be transplanted. She also noted that some accessions only flowered in October and could not be harvested. Since the trial had only recently finished the data are still being prepared for upload to the database. As noted previously, the trials will be repeated in 2023.

Z. Girek reported on her trials in Smederevska Palanka in 2021 and 2022. Due to late seed arrival, the trial accessions in 2021 were sown late and not under optimal conditions. Twenty accessions

showed significant heterogeneity in fruit shape and were accordingly split into different genotypes. Seeds had been harvested to be used in trials in 2022, although another 20 accessions did not flower or produce fruit under field conditions. The trial in 2022 was conducted at a different location and again had some accessions with poor germination and fruit set, suggesting that the climatic conditions are not ideal for all accessions. It will be interesting to analyze the data for an environmental effect. The datasets are currently being finalized and curated for upload to the EURISCO-EVA intranet.

Chiara Milanesi (Consorzio Sativa, Italy) reported on her trials conducted in the greenhouse in 2021 and 2022, with 60 and 56 accessions, respectively, evaluated. Seeds were sown in March and transplanted in May. Similar to other partners she noted low germination and heterogeneity for some accessions. Both datasets have been provided for curation and upload to the database.

Marta Fernandez Zurro (Semillas Fito, Spain) conducted greenhouse trials in Almería. The trial in 2021 included six plants each of 80 accessions, with three accessions not germinating and several accessions recorded as heterogeneous. Thirteen accessions that did not give good results in 2021 were included in the 2022 trial. The Field season in Spain goes from July to February, and data from the second trial will be available in spring 2023. She noted some problems with fruit setting, which may be due to the weather/environment or the material. Partners said that it may be interesting to identify material that has good fruit setting at high temperatures.

Enrico Belfanti (Tera Seeds, Italy) noted that he conducted a trial on all available material in 2021. The dataset has been provided and is currently being curated, as some trait scorings were unclear and would need further discussion.

Gelsomina Formisano (La Semiorto Sementi, Italy) noted that the trials in 2021 were not successful due to late sowing and the trial in 2022 had just been finalized. The dataset is in preparation and will be provided when ready.

Partner HAO Demeter had already provided both datasets for curation and upload, but could not be present at the meeting. Partner ESASem was also not present at the meeting and has not yet provided data and feedback from their trials in 2021 and 2022.

Sativa and Semiorto, as well as HAO Demeter, had so far only evaluated  $\frac{2}{3}$  of the available accessions and expressed interest to use 2023 to evaluate the remaining accessions for which seeds are available.

### **3. Data management**

During the morning meeting, participants visited the facilities of partner La Semiorto Sementi in nearby Sarno. After an introduction by General Manager Giuseppe Mancuso, participants toured the laboratory and warehouse and were able to watch seed processing in action. La Semiorto is a family-run business founded in 1970 and works on horticultural plants, flowers and aromatic herbs.

After this field visit, Suman Kumar (IPK, Germany) presented the EURISCO-EVA intranet in a hands-on session, demonstrating how to use the data uploader and providing examples for searching, filtering and displaying phenotypic data. He reviewed the final version of the data collection template, which had been modified to make data curation easier and allow partners to upload data themselves. User guides have been prepared for the uploader and the use of the web interface and should be consulted by partners as needed.

Partners appreciated the features of the database and provided suggestions for additional useful features. For example, it would be good if the chart function could display the distribution of trait scores across different trials for each accession, in order to see whether a certain trait is influenced by the environment. Similarly, it would be useful to display the variation of trait values over experiments.

Partners discussed how to curate data for heterogeneous accessions. In the agreed experimental protocol, instructions were given to record the predominant score, with the possibility to add details in a remarks field. This has been done by most partners, those who have not recorded as such will edit their data accordingly. Some traits have a score 'other' already defined, especially in flower traits. Partners agreed on heterogeneous material when the variation occurs with prevalence to record the main character, while when variation occurs without prevalence, they agreed to use 'other' value and provide details in the remarks.

Given the low number of datapoints, it was further agreed to create a trial for the marker genotyping dataset created by IGC in 2021. This would facilitate the comparison between resistance marker score and observed phenotypic data.

## 4. Data analysis

Partners discussed possible approaches for data analysis, noting, however, that in the absence of all finalized datasets this would be a theoretical exercise. A first approach was suggested to consider the different trial locations as replicates, also given that most accessions were not repeated in the same locations over two years, although this would mean that analysis of variance may not be possible in this case.

T. Cardi suggested that for each trait and trial, accessions should be ranked based on their trait scores and the ranking compared between the different trials. This would allow identifying variation within accessions for different traits as well as whether this variation is consistently observed in all locations.

Partners listed their priority traits, and main interests were identified as resistances, fruit characteristics, performance in different locations and also time of maturity. Pungency was noted as potentially being influenced by the environment and partners were encouraged not to use intermediate values when scoring pungency, as this could be very subjective.

It was proposed to analyze the datasets from greenhouses and open field trials separately, and to compare the performance of accessions between these different growing conditions.

Partners were reminded to doublecheck their datasets for any unreliable scores, and to upload only robust and reliable data to the database for further analysis. Where available, partners should also provide environmental and weather data for their trial locations, to identify any correlation of phenotypic data with environmental effects.

P. Tripodi will lead the analysis of genotyping data; CREA will also perform preliminary analysis of the phenotypic data to present to partners in spring 2023. In order to perform GWAS on the available *C. annuum* accessions, it will be important to ensure that the data are robust and display consistent variance in trait scores across experiments, and the decision on whether this is possible will be made after an initial analysis.

The most important issue, at this point, is to receive all datasets, properly curated according to agreed standards. Given that some trials will still be conducted in 2023, a full analysis including this data would be possible only in 2024.

## 5. Outlook – EVA Pepper 2.0

During the last session, partner shared feedback on the project so far and discussed ways to continue their collaboration in the future.

### 5.1 Dissemination and exploitation

S. Goritschnig reported on dissemination activities in the EVA project, where the EVA carrot network had presented preliminary results during the International Horticultural Congress 2022 in Angers, France as well as the International Carrot Conference in Mt. Vernon, USA. Another example of dissemination of project results is a paper currently in preparation to describe the novel SPET genotyping panel developed within the EVA Lettuce network, led by P. Tripodi.

P. Tripodi suggested that results from the EVA pepper network could be presented during the 2023 congress of the Italian Society of Genetics in Agriculture (SIGA), which will take place in September in Bologna. Partners were generally positive about sharing project results during scientific conferences, as long as the identity of resistant accessions or other interesting details would be anonymized.

It was generally recognized that the main exploitation of the materials and generated results would be done by project partners, especially breeding companies. Whether the results would also be amenable for dissemination in scientific publications will depend on the quality of the datasets and should be discussed again after initial data analysis.

### 5.2 Proposals for EVA Pepper 2.0 – continuation of network activities

S. Goritschnig presented a few considerations for a possible continuation of the network collaborations. The current project ends in November 2023, around the same time as the current Phase X of ECPGR. The work programme for ECPGR in its XI<sup>th</sup> phase is currently in preparation and will be discussed during the next Steering Committee meeting in June 2023. In general, given the positive feedback received from all EVA networks so far and the inclusion of evaluation initiatives in the [Plant Genetic Resources Strategy for Europe](#) published in 2021, some aspects of the EVA programme, such as coordination and the EURISCO-EVA database, may be considered for the regular budget of ECPGR. Additional network activities, e.g. genotyping, lab experiments and data analysis would certainly need external funding, which should be considered when reviewing the work plan for the next project phase. A stronger involvement of the ECPGR Working groups would be welcome to expand the genebanks involved in the network and gain access to additional materials. The EURISCO catalogue currently contains more than 11,000 bell pepper accessions of which less than 1,500 have associated evaluation data, from CGN and CRI (Czech Republic). In general, it may be good to include additional genebank and private sector partners in the network for a next phase.

Partners provided a round of feedback on their experience with the network so far.

W. v. Dooijeweert was happy to see the performance of CGN materials in multilocation trials and welcomed the additional information that adds to their collection. He is interested in continuing the collaboration and would also provide additional material for future accession sets. He proposed to look into heat and drought stress and water use efficiency as new traits of interest for the network.



Reza Darrudi (Semiorto) expressed their contentment with being able to explore old varieties and identify samples that may be useful for today's breeding. For small companies it is not easy to maintain their own collections and having access to genebank materials is therefore a clear benefit. Semiorto wants to continue the collaboration, keeping in mind that the work plan should be organized according to capacity of different partners and considering the time commitments for trait scoring. Chiara Milanesi seconded this observation, adding that the experimental plan could be revised to reduce workload where possible, especially for trait scoring that needs multiple observations. She also favoured focusing on implementing lab disease tests and abiotic stress responses.

M. Fernandes Zurro and Z. Girek also expressed interest in exploring more materials in future evaluations and noted that they appreciated seeing evaluation data from accessions in different locations.

K. Sarikyan noted her experience working with the Vavilov institute and AVRDC, and expressed interest in exploring the diversity of the material, also with a view to fruit processing. She noted that both *Verticillium* and *Fusarium* resistances are useful traits at her location and that the current collection seems to perform well for those two traits.

P. Tripodi questioned whether the focus of a future collaboration would be to continue more in-depth work on the existing collection (e.g. more traits, more trials) or whether it should be on exploring more genetic material. T. Cardi noted that first, the value of the current collection needs to be defined based on preliminary analysis and take a differential approach. For example, no resistance to Tomato spotted wilt virus (TSWV) has been identified in the collection, so more material should be screened. On the other hand, some ToBRFV resistant accessions have been found, for which it may be useful to continue more in-depth work. He noted the general feedback from partners who are interested in exploring additional genebank collections and wondered if it may be useful to develop a core collection based on genotyping data.

W. v Dooijeweert explained that CGN had used genotyping data from the G2PSol project for curation of their collection and was able to identify duplicates and misclassified materials. He advised against creating a core collection. In their online database, users are able to create their own core collection based on available passport data and using an online 'core selector'. In the future, they would also consider genomics data for this feature. He also questioned in which way the EVA materials would be made available for the community, noting that for the materials from G2PSol INRAE has taken charge of curation.

Partners were reminded that all material exchange in the project occurs under the terms of the Standard Material Transfer Agreement (SMTA) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), even though pepper is not included in Annex 1. This implies that all further material exchange should occur under the same conditions, that material is free to use in breeding and research and that upon commercialization of a new variety derived from genetic material in EVA, the partner should contribute to the Access and Benefit-sharing Fund of the ITPGRFA according to the current rules.

## 6. Conclusions of the meeting

T. Cardi expressed his satisfaction with the progress of the project so far and emphasized the need to strengthen the link between industry and the public sector. EVA is a good way to spread information on the work of ECPGR and to make genebank materials available to breeders. The positive feedback from the different networks shows that the project has established a functional system, which should be continued in the future. He noted that it would be good to include EVA in the ECPGR mandate, allowing funding to be dedicated to this project. In addition, it would be advisable to link the ECPGR grant scheme and EVA, in order to improve the interaction between Working Groups and EVA networks.

S. Goritschnig thanked all partners in person and online for their active participation in discussions and Semiorto for the interesting visit. An action list with next steps is included in Appendix 3. A virtual meeting will be planned for spring 2023 to review initial results and partners were reminded to provide their datasets as soon as possible to allow data analysis to start. A final in-person project meeting could be planned in late 2023 to work on the project continuation.

The meeting was adjourned.



EVA Pepper meeting group picture

## Appendix 1. Meeting agenda

### Agenda

Venue: Aequa Hotel, Vico Equense

<b>WEDNESDAY 26 OCTOBER</b>		
13:30 – 14:00	Registration	
	<b>Welcome and introductory session</b>	<b>CHAIR: T. CARDI</b>
14.00 – 14.10	Welcome by local host and ECPGR	<i>T. Cardi</i> <i>S. Goritschnig</i>
14.10 – 14.20	Introduction of participants	<i>All</i>
14.20 – 14.30	Overview of the current status of the ECPGR Evaluation Network EVA	<i>S. Goritschnig</i>
	<b>Results from EVA Pepper project</b>	<b>CHAIR: S. GORITSCHNIG</b>
14.30 – 14:40	Review of network workplan 2019-2023	<i>S. Goritschnig</i>
14.40 – 14:55	Review of selected accessions (criteria, previous knowledge, availability) and regeneration activities	<i>W. v. Dooijeweert</i> <i>P. Tripodi (online)</i> <i>Z. Girek</i>
14.55 – 15:40	Preliminary results of Pepper genotyping	<i>P. Tripodi (online)</i>
<b>15:40 – 16:10</b>	<b>TEA/COFFEE BREAK</b>	
16:10 – 16:30	Lab trials – reports from evaluating partners (~5-7 mins each)	<i>E. Dell'Olmo</i> <i>Y. Elkind</i>
16.30 – 17.00	Field trials – reports from evaluating partners (~5-7mins each)	<i>K. Sarikyan</i> <i>Z. Girek</i> <i>E. Belfanti</i> <i>C. Milanesi</i> <i>M. Fernandez Zurro</i>
17.00 – 18.00	Discussion	<i>All</i>
<b>20.00</b>	<b>SOCIAL DINNER</b>	

<b>THURSDAY 27 OCTOBER</b>		
<b>8:00 – 13:00</b>	<b>EXCURSION</b>	
	Visit to EVA Partner La Semiorto Sementi, Sarno (SA)	<i>G. Formisano</i>
<b>13:00 – 14:00</b>	<b>LUNCH</b>	
	<b>Data management</b>	<b>S. GORITSCHNIG</b>
14.00 – 14.20	Review of data collection templates	<i>S. Goritschnig</i>
14.20 – 15:30	Hands-on session with EURISCO-EVA intranet	<i>S.Kumar remotely</i>
<b>15.30 – 16.00</b>	<b>TEA/COFFEE BREAK</b>	

	<b>Data analysis</b>	
16.00 – 17.00	Brainstorming and discussion on data management and analysis –questions to be addressed, approaches, possible contributors	<i>All</i>
17.00 – 18.00	Planning of data analysis work for 2022/23	<i>All</i>
<b>18:00</b>	<b>FREE EVENING</b>	

<b>FRIDAY 28 OCTOBER</b>		
	<b>Outlook – EVA Pepper 2.0</b>	<b>CHAIR: T. CARDI</b>
09.00 – 10.00	Dissemination and exploitation of results	<i>S. Goritschnig</i>
10.00 – 10.30	Proposals for EVA Pepper 2.0 – continuation of network activities	<i>T. Cardi</i> <i>S. Goritschnig</i>
<b>10.30 – 11.00</b>	<b>TEA/COFFEE BREAK</b>	
11.00 – 12.00	Discussion on continuation of network activities after end of current project (Nov. 2023)	<i>All</i>
12:00 – 12.30	Wrap-up of meeting	<i>S. Goritschnig</i>
<b>12.30 – 13:30</b>	<b>LUNCH</b>	
13.30	End of meeting	

## Appendix 2. List of participants

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### Appendix 3. Action list

#	Activity	Action	Responsible	Due date
1	Field evaluations	Finalize datasets for trials from 2021 and 2022	partners	Dec 2022
2	Lab evaluations	Finalize datasets for trials from 2021 and 2022	partners	Dec 2022
3	Data management	Update trial list to reflect cancelled and additional trials	S. Goritschnig	Dec 2022
4	Data management	Facilitate upload of datasets to EURISCO-EVA	S. Goritschnig	Feb 2023
5	Seed distribution	Check availability of accessions of complementary seed sets for Sativa, Semiorto and HAO	S. Goritschnig/A. Brunazzi	Dec 2022
6	Seed distribution	Provide missing seeds to Sativa, Semiorto and HAO	A. Brunazzi	Jan 2022
7	Genotyping	Create Trial-ID and curate data for upload to EURISCO-EVA	S. Goritschnig	Dec 2022
8	Genotyping	Compile list of misclassified and heterozygous accessions	P. Tripodi	Dec 2022
9	Genotyping	Extract heterozygous accessions and compare with phenotyping observations.	P. Tripodi/S.Goritschnig	March 2023
10	Genotyping	Extract data from potentially misclassified materials to share with genebanks.	S. Goritschnig	March 2023
11	Data analysis	Compare germination data and dropouts from different locations	S. Goritschnig	March 2023
12	Data analysis	Perform initial data analysis on available datasets	CREA	March 2023
13	Data analysis	Compare identity of heterogeneous accessions with info available on CGN website	W. v. Dooijeweert	March 2023
14	Meeting	Organize virtual meeting to discuss preliminary results	S. Goritschnig	April 2023