

Summary report of the meeting

Contents

| | |
|---|----|
| 1. Welcome and introduction..... | 2 |
| 2. General discussion of the project | 2 |
| 2.1 Review of project proposal and new developments..... | 2 |
| 2.2 Current activities in EVA carrot | 2 |
| 3. Development of detailed evaluation protocol..... | 4 |
| 4. Selection of traits for evaluation | 5 |
| 5. Timeline and next steps | 6 |
| 5.1. Seed distribution to evaluators..... | 6 |
| 5.2. Experimental protocol and standard descriptors | 6 |
| 5.3. Data collection templates..... | 6 |
| 5.4. Cooperation agreement | 6 |
| 5.5. Subcontracts for evaluations..... | 6 |
| 5.6. Genotyping | 6 |
| 5.7. Passport information on accessions | 6 |
| 5.8. Regeneration of accessions..... | 7 |
| 5.9. Next meeting | 7 |
| Appendix 1. Meeting agenda..... | 8 |
| Appendix 2. List of participants | 10 |
| Appendix 3. Acronyms and abbreviations | 12 |

The meeting substituted for an in-person project meeting on 11-12 May 2020 in Angers, France, and took place on 11 May 2020, 12:30 to 16:30, 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 on MS Teams. These included presentations on ECPGR and the EVA networks and on EURISCO and its role within EVA.

1. Welcome and 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.

2. General discussion of the project

2.1 Review of project proposal and new developments

The EVA coordinator presented an update on the EVA carrot project, reviewing the project plan and informing of an extension of the project until November 2022, granted by the German donors in December 2019.

A draft workplan and timeline for the project were presented. The main tasks and activities involved in evaluating the carrot accessions were listed and partners were invited to confirm their roles and responsibilities as well as evaluation sites in a draft table. Partners were also informed that the project budget had been adjusted to distribute funds to recipient partners in accordance with the workplan, keeping total amounts designated for the EVA carrot project at the same level.

2.2 Current activities in EVA carrot

Thomas Nothnagel (TN; Julius Kühn-Institute (JKI), Quedlinburg, Germany) provided an update of ongoing activities. JKI is contributing to the project by providing evaluation for disease resistance traits in controlled environments, and relevant experiments have been initiated. Plants grown in the greenhouse will be phenotyped for 14 minimum descriptors before being included in resistance tests for *Alternaria alternata*, *A. dauci*, *A. radicina* and *Mycocentrospora acerina*, and the selected protocols were presented. Evaluations will make use of an in-house Lemnatec system for quantitative scoring, which has been successfully applied in the previous ECPGR project CarrotDiverse.¹ In response to a partner's question, TN clarified that among different available strains for each pathogen a strain with high aggressivity would be used and tested under controlled conditions, which should guarantee reliable results.

One partner questioned why the chosen protocol for *A. radicina* used root slices and not petioles. TN noted that the test on root slices provided more reliable data than that on petioles, which was considered outdated. He also agreed to share the data collection template that he intends to use for phenotyping, as this could be adapted for the other evaluations.

¹ [Improving the characterization and conservation of umbellifer crop wild relatives in Europe \(CarrotDiverse\)](#)

JKI will also collect tissue samples to be used in genotyping and offered to lead the genotyping data analysis, based on their experience within CarrotDiverse. Charlotte Allender (CA; UK Vegetable Genebank (UKVGB), Warwick, UK) expressed interest in contributing to the bioinformatics analysis of genotyping data. One partner noted that the genotyping and data analysis approach would need to be further discussed, as it should be clarified how to link data generated by genotyping by sequencing (GBS) to the genetic map of carrot in order to be able to develop molecular markers, and whether data should be generated on a single plant or bulked material. As genotyping is only planned for 2021 this discussion was postponed to the next meeting and TN agreed to sample tissue from individual plants and store it at -80°C until further notice.

TN noted the possibility of involving a collaborating laboratory in Berlin, the Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, who may be interested in contributing to the project with chemical analysis for polyacetylenes and volatile compounds, possible both for the greenhouse as well as the field trials. Partners agreed that these data could be interesting to collect, and could be included as an optional trait, but this would require further discussion and clarification.

During general discussions on project-related matters, partners were invited to indicate the dates for their planned field trials in 2020. Most partners confirmed their availability to do the trials as planned. Annette Hägnfelt (AH, Nordic Genetic Resource Center (NordGen), Sweden) indicated that they were not able to do field trials in 2020, because restrictions in response to the coronavirus pandemic had necessitated a redistribution of resources within NordGen. Trials for 2021, however, should be able to proceed as planned.

Partners discussed plans for regeneration of accessions as proposed in the project, noting that the accessions in the evaluation were selected based on availability of sufficient seed. Therefore, the goal is to ensure that all evaluated accessions are available in genebanks after the project, thus potentially necessitating regeneration. Emmanuel Geoffriau (EG) invited partners to review the list of accessions which included additional lines available in EURISCO, especially cultivated landraces, which should be regenerated and asked genebanks to note their capacity for such regenerations, inviting also companies to contribute to this effort.

NordGen is moving their basic seed storage facilities so it is important to order seed as soon as possible. Since an ongoing project of minimizing the backlog that NordGen has regarding regeneration, our personnel and other resources are fully utilized. NordGen has therefore currently no capacity for regeneration. CA noted that UKVGB usually starts their regeneration in August each year with seed harvest scheduled in autumn of the following year. She added that they prioritized identification of new landraces for future experiments and could add 1-2 lines for the EVA project. Violeta Lopes (Banco Português de Germoplasma Vegetal (BPGV), Portugal) said that their collection of landraces is relatively small and that their focus for regenerations next year would be on wild accessions.

Arnaud Thabuis (Rijk Zwaan, France) informed partners that they would perform an additional trial for flowering traits interesting for breeding and seed production in their field station in France in 2020. Seeds for this experiment would be sown later in the season and have been accounted for in the seed amounts requested from the genebanks.

3. Development of detailed evaluation protocol

EG led the discussion, presenting a draft experimental protocol which should provide guidance to all field evaluators. He highlighted the need for assigning a specific EVA code to each accession, which would allow effective data management and ensure that a good blind trial is guaranteed. The proposed coding was adapted from the one already in use by TN in their trials at JKI.

The choice of appropriate controls was noted, and the need to use untreated seeds. Two main controls will be provided to all evaluators and shipped together with the accessions: one early variety which is less pervious to disease pressure (Yukon, Syngenta) and one late variety, which is resistant to *Alternaria* (Maestro, Vilmorin). EG further confirmed that his lab would collect the seed shipments from the participating genebanks, aliquot and redistribute all to the evaluators. AH reminded evaluators that seeds from the genebanks have been extensively dried for long-term storage and recommended to maintain them at ambient temperature for a few days before sowing to improve germination.

Discussing the trial setup, it was noted that it would be impossible to completely standardize field trials as they depend on existing local conditions, but whatever possible should be harmonized, e.g. setting the harvest date at 120 days would guarantee a single evaluation cycle for all.

Some partners cautioned that the ideal harvest time could be very different for evaluations in the south vs. north of Europe and suggested to do one replicate at the ideal harvest time and one at the 120-day timepoint. AH recalled that typically the length of a carrot is defined at 6-7 weeks, whereas anthocyanin and carotene accumulation happen late in development, closer to the 120-day timepoint. One partner suggested that the ideal harvest time would depend on the traits, noting that quality-related traits should be evaluated at defined dates.

Evaluation partners provided information about their evaluation sites and expected sowing dates for 2020, guaranteeing nine locations in five countries.

The partners agreed to use a 2-block experimental design with two repetitions per accession, where possible. Partners agreed to randomize their plots and to use the previously agreed EVA codes. TN noted that he could update information on germination rates of the selected accessions based on their ongoing experiments, which was welcomed as germination tests by genebanks are usually done in vitro and not in soil.

Partners discussed the use of crop protection during the trial. Application of pre-emergence herbicide at low level was deemed necessary to prevent excessive growth of weeds. As this may affect germination of some accessions, it was suggested to increase the number of seeds sown for these with lower germination rate. One of the goals of the project was to evaluate naturally occurring diseases, therefore partners agreed that pesticide treatment during the trials should be avoided and only applied if the whole trial was jeopardized. Partners agreed that systematic treatment should be avoided at all cost, however, depending on disease pressure, early treatments may be necessary to save the trial but would still allow data from later timepoints to be collected.

In order to correlate evaluation and environmental data, climatic data (e.g. temperature, radiation, rainfall) should also be collected. One partner suggested to use data from the JRC database (<https://agri4cast.jrc.ec.europa.eu/DataPortal/Index.aspx>), which collects this information at a

fairly precise level, which would allow harmonization of data across all evaluation sites in Europe. In addition, partners were invited to note as much as possible detailed information on the crop management practices, including units of fertilizer, irrigation amount and input of crop protection.

4. Selection of traits for evaluation

EG introduced a draft list of characterization and evaluation traits which should be scored by partners during the field trials. They were grouped in traits to be observed in the field, traits to be observed at harvest and traits to be observed post-harvest and distinguished between evaluation traits more interesting for breeders and morphological characterization traits (collected from IPGRI descriptors and the minimal descriptor list compiled by the ECPGR Umbellifer Crops Working Group)². Partners reviewed the list, discussed the relevance of each proposed trait, the frequency of observation and the scale for the scoring. Partners also reviewed the list with respect to the data embargo requirement and agreed on which traits (mainly descriptive characterization data) would be collected outside the EVA embargo and thus be directly made available in EURISCO.

Evaluation traits observed in the field would focus on naturally occurring diseases and bolting tendency. Partners suggested to include pictures in the protocol as references to facilitate assessing disease severity. Homogeneity across the plot was not considered a necessary trait to be scored as at low disease pressure it may be difficult to distinguish between actual resistance and the material. It was suggested to collect data at specified time points during the growth phase, noting that it would depend on the disease assessed. One partner suggested to add an observation at an earlier time point to score vigour of emergence, which was included as an optional trait in the list.

Characterization data to be collected in the field cover IPGRI descriptors such as leaf morphology, height and growth habit.

Evaluation data to be collected at harvest included visible pathogen damage and other quality traits. Pictures were again requested to accompany the protocol to facilitate scoring. It was noted that the harvest date should be clearly specified in the protocol, although scoring of traits may take several days in the field. Scoring of quality traits requiring specific equipment were considered optional, as these could provide interesting information but not every partner was able to observe them.

Characterization data to be collected at harvest include minimal descriptors for root size, morphology and pigmentation. Partners agreed to complement the data with pictures of harvested carrots.

Partners agreed that evaluation data for post-harvest traits should be collected optionally, as it had not been discussed previously and not every evaluator was equipped to store the harvested material for several months.

² [Minimum characterization descriptors for carrot](#) (Appendix II in Report of the Second Meeting of the Umbellifer Crops Working Group, 26-28 June 2013, St. Petersburg, Russian Federation. IPGRI. 1998. [Descriptors for wild and cultivated Carrots](#). International Plant Genetic Resources Institute, Rome, Italy.

Partners noted that standard protocols including reference pictures as well as data recording templates should be finalized as quickly as possible because field trials would be starting in June.

5. Timeline and next steps

Based on decisions made in the meeting the following activities will be coordinated:

5.1. Seed distribution to evaluators

Sixty genebank accessions as selected previously will be dispatched by the holding institutes to Emmanuel Geoffriau (Agrocampus Ouest, Angers, France), who will coordinate the aliquoting and redistribution of accessions, including appropriate controls, to evaluators.

5.2. Experimental protocol and standard descriptors

The experimental protocol will be finalized to reflect decisions made during the meeting. The traits list will be complemented with appropriate descriptors, scales and photographs, as necessary. Partners are requested to supply photographs where available.

5.3. Data collection templates

As discussed, templates for data collection need to be developed and finalized as quickly as possible, because field trials are commencing in June 2020. These should facilitate recording of all relevant information of the experimental setup as well as scoring of traits according to the agreed standard descriptors. The data template provided by TN will serve as a basis for this.

5.4. Cooperation agreement

Partners were informed that the draft agreement presented in this meeting is currently under revision in order to simplify and generalize the description of tasks and to clarify the embargo period. This agreement should have been ready and signed by all partners before evaluation trials begin.

5.5. Subcontracts for evaluations

Several public research institutes participate in the evaluation trials and are supported by project funds. Subcontracts for these activities need to be signed between the ECPGR Secretariat and the respective partners.

5.6. Genotyping

While not urgent, several decisions need to be taken and relevant information collected. Quotations from commercial providers for the selected genotyping method should be requested, and sampling or bulking of material needs to be considered. Genotyping data analysis will be based on experience from CarrotDiverse and approaches are described in the reports of this project.

5.7. Passport information on accessions

Providers of GR material should provide as much information on the accessions they shared as possible (origin, existing characterization data, EURISCO identifier, etc.). A template to gather this information will be developed by the ECPGR Secretariat and EURISCO coordinator and shared with genebank curators to be completed by them.

5.8. Regeneration of accessions

Genebanks were asked to indicate which accessions they are regenerating as part of the EVA project. Because this activity is partially supported through project funds, subcontracts for these activities need to be made between the ECPGR Secretariat and the respective partners. Companies willing and able to regenerate carrot accessions in kind should indicate this in the table so that these activities can also be planned.

5.9. Next meeting

It was proposed to convene an online meeting on 4 June 2020, before the start of evaluations. During this meeting final operational questions on field trials, standard protocols and data collection should be clarified and the cooperation agreement reviewed. Future discussions should also address data analysis methods, reporting and other open questions related to evaluation, regeneration and genetic material.

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 carrot component | S. Goritschnig |
| ppt | EURISCO: ensuring integration of data in special intranet environment for EVA | S. Weise |
| Documents | Drafts available for review: <ul style="list-style-type: none"> • EVA carrot proposal (updated) <ul style="list-style-type: none"> - Workplan 2020/2021 - Roles and responsibilities of partners - EVA carrot budget allocations - EVA carrot trial protocol • Cooperation Agreement | S. Goritschnig / E. Geoffriau |

11 MAY, 12:30– 16:30 (Venue: MS Teams)

| | | |
|---------------|---|-----------------------------|
| 12:30 – 13:00 | Connecting to MS Teams – technical assistance if needed | |
| | Welcome | |
| 13:00 – 13:05 | Welcome and introduction of platform and available files/tools | S. Goritschnig |
| 13:05 – 13:15 | Introduction of participants | All |
| | General discussion of the project | Chair S. Goritschnig |
| 13:15 – 13:30 | Review of project proposal and general Q&A on pre-meeting documents | S. Goritschnig |
| 13:30 – 13:45 | Update on current activities in the EVA network Carrot – Lab tests | T. Nothnagel |
| 13:45 – 14:30 | Discussion on: Regeneration of accessions Seed processing and distribution Management of Evaluation data (Embargo) Roles and responsibilities of partners Workplan 2020/2021 Contingency planning due to coronavirus | All |
| 14:30 – 14:45 | Break | |

| | Development of detailed evaluation protocols | Chair: E. Geoffriau |
|---------------|--|----------------------------|
| 14:45 – 15:15 | Ontology and practical protocol for field traits to be evaluated (diseases, bolting ...) | All |
| 15:15 – 15:45 | Ontology and practical protocol for traits to be evaluated at harvest and postharvest (diseases, pests, physiological disorders...) | All |
| 15:45 – 16:15 | Ontology and practical protocol for carrot quality traits to be evaluated (Refractometer index, pigments...) | All |
| 16:15 – 16:30 | Wrap-up of meeting: Overview of cooperation agreement, timelines and deliverables Define next steps Next meeting (as necessary): 4 June 2020, 13:00 – 16:30 | S. Goritschnig |

Appendix 2. List of participants

Charlotte Allender
University of Warwick, School of Life
Sciences
Wellesbourne Campus
Warwick CV35 9EF
United Kingdom
Tel: (44) 24 7657 5014
Email: charlotte.Allender@warwick.ac.uk

Emmanuel Geoffriau
Agrocampus Ouest Angers INHP
2 rue le Nôtre
49045 Angers
France
Tel: (33) 241225431
Email: Emmanuel.Geoffriau@agrocampus-
ouest.fr

Micha Groenewegen
Living Seeds - Sementes Vivas
Herdade do Couto da Várzea
Estrada Nacional 354
6060-270 Idanha-a-Nova
Portugal
Tel: (351) 910453855
Email: loja@sementesvivas.bio

Annette Hägnefelt
Nordic Genetic Resource Center (NordGen)
Smedjevägen 3
230 53 Alnarp
Sweden
Tel: (46) 760063425
Email: annette.hagnefelt@nordgen.org

Aurélie Ingremeau
OBS Innovation
2 Kernonen
Plougoulm
France
Tel: (33) 0298299255
Email: aurelie.ingremeau@o-b-s.com

Violeta Lopes
Banco Português de Germoplasma Vegetal
(BPGV)
Quinta de S. José, S. Pedro de Merelim
4700-859 Braga
Portugal
Tel: (351) 253 198730
Email: violeta.lopes@iniav.pt

Cristina Mallor Gimenez
Unidad de Tecnologia en Producción
Vegetal
Avda. Montañana 930
50059 Zaragoza
Spain
Tel: (34) 976713078
Email: cmallor@aragon.es

Thomas Nothnagel
Julius Kühn-Institut (JKI) - Federal
Research Centre for Cultivated Plants
Erwin-Baur-Str. 27
06484 Quedlinburg
Germany
Tel: (49) 394647430
Email: thomas.nothnagel@julius-kuehn.de

Paolo Pagan
CAROSEM GmbH
Maschweg 105
49152
Germany
Tel: (49) (5472) 1353
Email: paolo.pagan@carosem.eu

Julien Sacré
Limagrain Vegetable Seeds
Centre de Recherche la Costière
30210 Lédenon
France
Tel: (33) 631331408
Email: julien.sacre@vilmorin.com

Sylvie Salgon
Takii France
660 chemin de la Crau, quartier de la
Malgue
13630 Eyragues
France
Tel: (33) (0)4 90 92 81 86
Email: s.salgon@takii.fr

Arnaud Thabuis
Rijk Zwaan France S.A.R.L.
La Vernède
30390 Aramon
France
Tel: (33) 4 66 57 37 64
Email: athabuis@rijkszwaan.fr

Stephan Weise
EURISCO Coordinator
Leibniz Institute of Plant Genetics and Crop
Plant Research (IPK)
Corrensstrasse 3
06466 Seeland
Germany
Tel: (49) 394825744
Email: weise@ipk-gatersleben.de

ECPGR Secretariat

Sandra Goritschnig
Bioversity International
Via dei Tre Denari, 472/a
00057 Maccarese
Rome
Italy
Tel: (39) 06 6118 313
Email: s.goritschnig@cgiar.org

Appendix 3. Acronyms and abbreviations

| | |
|---------|--|
| ECPGR | European Cooperative Programme for Plant Genetic Resources |
| EURISCO | European Internet Search Catalogue |
| EVA | European Evaluation Network |
| GBS | genotyping by sequencing |
| JKI | Julius Kühn-Institute, Quedlinburg, Germany |
| NordGen | Nordic Genetic Resource Center, Sweden |
| UKVGB | UK Vegetable Genebank, Warwick, UK |