

**Summary report
of the**

**Annual meeting of the
European Evaluation Network (EVA) for Carrot**

**2 October 2023
York, United Kingdom**



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The 2023 Annual Meeting of the EVA Carrot Network was held in person in York, United Kingdom. Partners who were not able to travel to the meeting connected online in a hybrid form. The agenda of the meeting is attached as [Appendix 1](#) and the list of participants as [Appendix 2](#).

1. Welcome and introduction

The EVA Coordinator, Sandra Goritschnig, opened the meeting, which was planned in conjunction with the '[ISHS International Symposium on Carrots and other Apiaceae](#)'. She welcomed participants, noting that partners from JKI had missed a flight connection and would only join in the afternoon. She reviewed the agenda and reminded participants of the expected outcomes of the meeting, which included a discussion of the current results, the development of a detailed work plan for the project until December 2024 and a proposal for the continuation of the network after the end of the current funding period.

1.1 Overview of the current status of the ECPGR Evaluation Network EVA

After a round of introductions, S. Goritschnig updated participants on developments within the EVA carrot and other networks. The EVA Carrot network currently has 14 partners from eight countries, including eight breeding companies. EVA partner Carosem has recently been acquired by Enza Zaden, who are starting their carrot breeding programme at a station near Angers, France. They had expressed interest in participating in EVA Carrot and will be contacted to officially join the network for the next phase.

During a general project update, S. Goritschnig reminded participants of a project and budget extension granted by the donor at the end of 2022, which allows the current project to run until December 2024 and provides additional funds to support data analysis and communication activities and to implement a SNP viewer to display genotypic data. Partners were asked to provide video material for use in the EVA promotional video currently under preparation. Furthermore, in a recently granted budget revision, additional ~€50,000 were made available by the donor for use in 2023, contributing to additional experiments on pepper and carrot, improving data management in EURISCO-EVA and the organization of a project meeting for the preparation of a future EVA Legumes network. This new EVA Legumes network is currently under development through the grant scheme activity '[Fostering the need of implementation of the ECPGR European Evaluation Network \(EVA\) on Grain legumes](#)' (ForEVA), of the ECPGR Grain Legumes Working Group (WG) under the leadership of WG chair Creola Brezeanu. This project, which has 23 project partners (mainly genebank curators and researchers), has so far attracted more than 35 stakeholders (private and public sector breeders and researchers) and met in Bucharest on 10-11 October 2023 to develop a work plan and budget for the future EVA Legumes network, which is due to start in 2024. In 2024, ECPGR is also starting a new Phase XI, and the EVA networks have been firmly integrated into the ECPGR work programme for the next five years, with guaranteed funding for coordination and EURISCO support until 2026.

In terms of dissemination of project results, partners were informed of the recently published article by Tripodi *et al* in *Frontiers in Plant Sciences*¹, describing the work of the EVA Lettuce network. The article submitted by EVA Carrot to *Acta Horticulturae* for the proceedings of the ISHS congress in 2022 is in production and publication may still happen in 2023. The EVA Carrot

¹ <https://doi.org/10.3389/fpls.2023.1252777>

network (Emmanuel Geoffriau, Institut Agro, France) will present the same poster as last year during the upcoming symposium and Holger Budahn (JKI) will give a presentation that will also include some data from the EVA Carrot work.

2. Results from EVA Carrot project

2.1 Review of network work plan 2019–2024

S. Goritschnig provided an overview and summary of the work plan agreed for the EVA Carrot network. Sixty carrot accessions and relevant controls have been evaluated in 26 field, greenhouse and lab trials over two years in 2020 and 2021. Genotyping was done using three different approaches, genotyping-by-sequencing (GBS), whole genome resequencing (WGS) and a SNP array. An additional dataset will be generated for metabolite analysis of the GBS genotyped plants, using samples collected at JKI and extra funding secured for 2023. With all phenotypic data on this first set being finalized and uploaded to the EURISCO-EVA intranet, the remainder of the project should focus on the joint data analysis.

Around 30 accessions have been regenerated for potential future use. INIAV are currently conducting regeneration activities of 15 carrot accessions: three wild (*Daucus carota*) and twelve landraces (*D. sativa*), with preliminary characterization of five accessions available and underway for the rest. Charlotte Allender noted that the UK Vegetable Genebank (UKVGB) is expecting an increase in stable funding from the government which may allow them to regenerate ~40 accessions per year, with a focus on landraces. Regenerations in the UK are typically sown in September. E. Geoffriau reminded partners that for evaluations a minimum yield of 26g of seeds should be achieved in regenerations.

The action points specified during the last project meeting in September 2022 were reviewed and open action points highlighted for partners. The EURISCO-EVA intranet database will be updated with relevant metadata, including for genotyping experiments, accessions (photos and descriptions) and trials (including summary files of photos taken during experiments).

As discussed during the 2022 meeting, five of the most representative photos of accessions should be selected to be included as metadata of the carrot accessions. Filippo Guzzon prepared a summary of the available photos taken during the different trials. Partners noted the variable quality of the pictures and agreed that a standard protocol and background poster with a blue colour background and a colour palette should be prepared to generate pictures that could be useful in automated image analysis. Participants agreed to include photos from 1) the 2020 trial in Angers (roots outside, inside), 2) the 2020 trial in Zaragoza (cut roots and shoulders), 3) the 2020 trial in Ledenon (roots outside and cut), 4) the 2021 trial in Zaragoza and 5) the 2021 trial in Angers (foliage). Partners also suggested that the database should allow filtering images by trial, trial location and accessions.

2.2 Updates on data analysis progress

Participants reported on their progress in analyzing data according to the task list agreed upon at the last annual meeting.

E. Geoffriau recalled the work of his master's student Delphine Hedoin who did a very detailed statistical analysis of the phenotypic data from all field trials. Her thesis and presentations are

available for partners on the SharePoint. The final dataset, processed for her analyses is also available and can be used for further studies.

Holger Budahn updated on progress with the genotyping analysis. Comparison of the used approaches showed variable coverage of the chromosomes, with GBS having the lowest coverage (0-181 SNP/Mbp), similar to the SNP array (20-143 SNP/Mbp) and WGS providing the most data (3,679-30,391 SNP/Mbp). Discriminant analysis separated the samples into three clusters, with the Spanish landraces forming a distinctive cluster. Genetic distances within accessions were calculated from GBS data and showed a relatively narrow genetic basis with values below 0,25 overall. Further analysis will look into the genetic components differentiating the Spanish accessions and performing association analyses for relevant traits. The data will be prepared for submission to a public repository and linked to the EURISCO-EVA database, and the datasets will also be prepared for implementation of a SNP viewer instance. Participants commented that a new version of the carrot genome was recently published² and suggested that it may be useful to remap the data to the new genome. Additional suggestions for analyses were to correlate molecular data with cultigroups and to investigate how to use GWAS data to create a core collection in heterozygous crops, as has been done for cabbage.

Thomas Nothnagel shared his update on the analysis of phenotypic data of the JKI trials. He noted that thanks to some additional funding, samples collected of ten plants per accession that were both extensively phenotyped and GBS genotyped will now also be analyzed for metabolite content, in collaboration with the University of Leipzig. These data should be available by the end of 2023 and will be very interesting as they can be used in GWAS analysis for metabolite production. The data for morphological traits have been collected both as a means of ten plants (for upload into EURISCO-EVA and ultimately the EURISCO database) but also for individual plants. Disease data were collected with image analysis and all data are now available in the database, except for *Mycocentrospora*, where there was a software problem in calculating disease values. All accessions have also been tested for *Meloidogyne hapla*. This test will be repeated next year using funding for another project, which will require some more seeds from all accessions (approx. 50 seeds).

In the lab trials, a large number of detailed photos were taken. While these are not representative to describe the accessions (as plants were grown in pots), it will be good to add them to the database as trial supplemental files. Two pathogens (*Alternaria* and powdery mildew) were evaluated both in the lab and field, and it will be interesting to compare the results. In the lab trials also some accessions from the CarrotDiverse project were used, to provide overlap which could allow comparison of the two datasets. For example, the GBS genotyping analysis could be done with both datasets together and mapped to the new carrot genome, this would increase the sample number and improve association analyses. It was noted that the CarrotDiverse activity report is still pending and would need to be submitted if the Umbellifer WG would like to apply for funding from the ECPGR Grant Scheme.

C. Allender outlined her plan for the diversity studies and comparisons with other studies, which includes a literature review of diversity studies, collection of available datasets and comparative analysis, and could work on this together with JKI, involving also the University of Warwick bioinformatics unit. She provided an outlook where additional funding for the UKVGB will probably

² <https://doi.org/10.1038/s41477-023-01526-6>

allow low coverage resequencing of genebank accessions, which will be selected in consultation with stakeholders. These data may be used to pool duplicate genotypes in order to optimize genebank resources. However, it will be important to gather all relevant information on each accession (also using AI and DOI) before pooling lines. It was highlighted that pooling would be applied only to breeding lines, not to PGR conservation.

E. Geoffriau presented an analysis of the classification of carrot in types and cultigroups. The goal was to use phenotypic data to predict the type of a certain carrot accession. Types are usually described based on colour and morphology (especially root shape). Considering that many landraces have mixed types in their populations it may be useful to create cultigroups that they could be assigned to. He also suggested, especially for landraces, to identify morphotypes instead of cultitypes as they are more inclusive, taking into consideration multiple morphological traits. Cultitypes are defined based on CPVO standards. He used linear discriminant analysis (LDA) with a reference training set to predict typology of the rest of the collection. Prediction was not always confirming the cultitype assigned by the genebank curator based on morphology traits, but this could also be due to the selection of accessions in the training set. A paper utilizing image analysis to use morphologies for prediction of cultitypes has been published by the United States Department of Agriculture (USDA). It was noted that clustering analyses based on phenotypic data did not reflect the predicted cultitypes, and suggested that using a smaller dataset with only the most informative traits might improve the clustering of cultitypes.

He also presented an analysis of the effect of plant density on root traits, which should be considered as a covariable to allow analysis of comparable datasets and define a homogeneous distance that should be preferred in trials. Partners discussed how to ensure consistent plant density, based on germination and subsequent thinning, and what traits may be affected by density. They considered that there may be a threshold for a density effect, which is likely different for above- and belowground organs. Marie Hardy volunteered to work on creating a model to correct trait data for density. They also highlighted the effect of density and weather on disease severity, as a minimum plant density is usually needed for disease progression in a field.

Juliette Chevalier presented initial analyses of root traits, using different methods to visualize the trait scoring across different trials, which facilitates the identification of patterns and outliers. She used the dataset provided by D. Hedoin and noticed some inconsistencies with the data in the database. Going forward, she suggested using the visualization via heatmaps and boxplots to help define which datasets of traits of interest to use for further analyses. One question that can be addressed with the data is to see if morphotype predictions match the root phenotypes observed.

Arnaud Thabuis presented an analysis of the flowering trial conducted by Rijk Zwaan in France. The carrot collection generally showed good distribution of values, with high heritability calculated for most traits and good correlation between traits observed for the primary and secondary umbels. A negative correlation was observed between seed set and flowering time, while seed set was positively correlated with morphological traits such as plant height or umbel size. Using the SNP array genotyping data he performed GWAS and found potential QTLs for some flowering traits; however, the small population size prevented the identification of statistically significant associations for all traits, especially those likely of a polygenic nature. Although some correlations were identified, a true proxy for predicting seed set was not identified. It was suggested to

determine the thousand kernel weight of the populations, as the seed set had been scored only qualitatively.

Sylvia Salgon presented an analysis of the correlation between lab and field trials for morphological traits. Root length showed a good correlation between the lab and most field trials, while root diameter was less well correlated, likely reflecting different growing conditions and differences in plant density. The ratio between root length and diameter could be used as a parameter to compare the different trials. She also noted that the control accessions had different scores for leaf growth habit across different trials, indicating that there is an environmental effect or it may be difficult to score correctly.

3. Planning of EVA Carrot activities in 2024

Participants discussed priorities and preferences for future EVA Carrot activities. A survey has been shared with partners to collect their input on priorities for work planning in 2024 and beyond in EVA Carrot 2.0. In general, respondents rated their experience so far very positively and expressed commitment to continuing the collaboration.

Work for 2024 should focus on finalizing data analysis to effectively valorize and exploit results from the evaluations so far, which have produced a large amount of data. Some regenerations should also be planned to feed into the next sets of accessions for evaluations, since this takes a while. Again, companies noted their availability to contribute to this task.

The visual representation approach for data presented by J. Chevalier was considered useful to allow the sorting of trials and materials according to their trait scores and the identification of outliers (both in material and trials). Only data from trials that show useful distribution should be used in comparisons. To study the GxE effect on resistances, only trials with sufficient disease pressure should be considered. As such, reducing the datasets to avoid too much variation may improve the significance of analysis results. Analyses should also focus on priority traits such as root shape or bolting tendency to enable planning of future trials. The R scripts developed by D. Hedoin in her Master's thesis could be applied to different trait data.

The data in the database will be completed with the missing data from lab trials to also allow comparison with those. E. Geoffriau agreed to analyze morphotype distribution in more detail and will work together with a statistics colleague on comparing genotypes and morphotypes and suggested employing another MSc student for additional analysis. S. Goritschnig confirmed that there are funds available to support data analysis from the EVA budget. C. Allender agreed to work on the analysis of Brix data.

Some ideas for possible publications of project results were collected, noting that all data should be available for use in publications: 1) comparison of multiple traits with qualitative and quantitative data using statistical model AMM, how to mix different types of data (E. Geoffriau, D. Hedoin's thesis); 2) diversity of carrots base on types, what criteria differentiate accessions (E. Geoffriau); GxE effects using AMMI modelling; 4) carrot development, linking root morphology traits and climate during development; 5) comparison of genotyping methods; 6) GWAS on phenotypic data from JKI lab trials.

A. Thabuis suggested describing the impact of the EVA network on the participating genebanks. This could include identifying which traits are more relevant to allow more efficient

characterization of genebank materials, developing a smart approach to use genebank materials more efficiently in trait discovery and finding the literal needle in a haystack.

4. Outlook – EVA Carrot 2.0

In order to plan for future trials, it will be important to know traits' heritability scores, to identify the most suitable locations and create subsets of trials to improve collaboration and better share efforts. Creating a shortlist of traits that are most important for breeders or describing accessions would allow the inclusion of up to 200 accessions in trials for which standardized pictures could be taken for further analysis. Using standard pictures could allow scoring morphology traits more objectively. Another suggestion was to focus a new set of accessions on certain types of materials or cultigroups, discarding bolting or very heterozygous landraces, as these tend to skew the analyses.

Partners agreed to extend the EVA Carrot consortium, especially to genebanks with interesting collections and research institutes that could provide specific expertise. A closer involvement with the ECPGR Umbellifer WG would be good. Some EVA partners have been not very responsive and new partners should be ready to actively contribute in-kind. As noted previously, cost-intensive experiments would need to be funded externally.

In terms of future carrot materials, partners favoured exploring more the European diversity of cultivated carrot, which is still understudied. C. Allender noted that historic data from a previous genetic resources project were available for valorization but needed to be processed to make the data accessible in the public domain. Partners agreed that prior knowledge of landraces could be valuable and considered when selecting material for future trials. Similarly, the data from CarrotDiverse on wild carrots still has not been fully analyzed and valorized, and finalizing this activity would be a prerequisite to including wild materials in the planning of EVA Carrot activities. E. Geoffriau noted that he had developed selfed carrot lines which could be useful for further studies.

Ideas for research questions to be addressed in EVA Carrot 2.0 expanded the current approach of characterization of genebank material, which should continue in a more effective way based on results of the current project. Priority traits are biotic and abiotic stresses and also nutritional value of carrots. Ensuring the connection to genotypic data was emphasized, as was the interest to do more in-depth analyses of the genetic background of traits of interest. The current structure of the EVA networks effectively splits evaluation activities into two levels, 1) field trials provided as in-kind contributions, and 2) genotyping and controlled environment experiments (e.g. nutritional analyses, abiotic stress trials) requiring extra funding. At its current funding level, the EVA Carrot network should therefore focus on evaluations in field trials, while other activities should seek to be funded by projects on national or international level. Partners highlighted their interest in collaborating on more risky projects and company representatives reminded that they were ready to contribute their expertise and capacity where possible.

Next steps to be organized are outlined in the action list in [Appendix 3](#). Most urgently partners should continue analysis, preparing to share results in a virtual meeting in spring 2024 and discuss their further development into publications. Potential new partners should be contacted and availability of carrot landraces surveyed among European genebanks. Another online survey among partners should identify priority traits.

5. Close of the meeting

S. Goritschnig thanked participants for their active participation and the efficient strategic discussions during the meeting and wished partners a successful Carrot Symposium.

Appendix 1. Meeting agenda

| | | |
|-------------------------|---|------------|
| SUNDAY 1 OCTOBER | | |
| 20.00 | SOCIAL DINNER AT RADISSON HOTEL YORK | <i>ALL</i> |

Venue: Radisson Hotel York

| | | |
|-------------------------|--|-------------------------------------|
| MONDAY 2 OCTOBER | | |
| 08:15 – 08:30 | Registration | |
| | Welcome and introductory session | CHAIR: S. GORITSCHNIG |
| 08.30 – 08.45 | Welcome by ECPGR | <i>S. Goritschnig</i> |
| 08.45 – 09.00 | Overview of the current status of the ECPGR Evaluation Network EVA | <i>S. Goritschnig</i> |
| | Results from EVA Carrot project | CHAIR: E. GEOFFRIAU |
| 09:00 – 09:10 | Review of network workplan 2019-2024 | <i>S. Goritschnig</i> |
| 09:10 – 10:00 | Updates on data analysis progress by various partners, as assigned during meeting 2022 <ul style="list-style-type: none"> • Results of Carrot genotyping, diversity structure of accessions - <i>H. Budahn</i> • Lab trials and GWAS - <i>T. Nothnagel</i> • Diversity studies (spanish accessions, EVA and comparison with other studies) - <i>C. Allender</i> • Classification of EVA accessions in carrot types - <i>E. Geoffriau</i> | |
| 10.00 – 10:20 | TEA/COFFEE BREAK | |
| 10:20 – 12:00 | Updates on data analysis progress and perspectives by various partners, as assigned during meeting 2022 <ul style="list-style-type: none"> • Root traits general analysis - <i>J. Chevalier</i> • Correlations between lab and field results for morphological traits <i>S. Salgon</i> • Location effects (bolting etc.) - <i>E. Geoffriau</i> • Flowering traits (GWAS, seed set proxy) - <i>A. Thabuis</i> | |
| 12:00 – 12:30 | Update of data analysis workplan for 2024 Dissemination and exploitation of results: <ul style="list-style-type: none"> • Communications in symposia • Publication planning | <i>all</i> |
| 12:30 – 13.30 | LUNCH | |
| | Outlook – EVA Carrot 2.0 | CHAIR: E. GEOFFRIAU |
| 13.30 – 13.45 | Proposals for EVA Carrot 2.0 – continuation of network activities | <i>E. Geoffriau, S. Goritschnig</i> |
| 13.45 – 15.00 | Development of work plan for EVA Carrot 2.0 | <i>S. Goritschnig, all</i> |
| 15.00 – 15:20 | TEA/COFFEE BREAK | |
| 15.20 – 17.00 | Development of work plan for EVA Carrot 2.0 | <i>All</i> |
| 17:00 – 17:30 | Final discussion and wrap-up | <i>All</i> |
| 17:30 | End of meeting | |

Appendix 2. List of participants

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Appendix 3: Action list

| # | Action | Responsible | Due by |
|----|--|--------------------|------------|
| 1 | Finalize curation of JKI lab trials datasets | F. Guzzon | 30/11/2023 |
| 2 | Determine TKW of flowering trial and update database | A. Thabuis | 31/12/2023 |
| 3 | Provide additional 50 seeds of EVA Carrot accessions to T. Nothnagel for <i>M. hapla</i> trials in 2024 | E. Geoffriau | 31/12/2023 |
| 4 | Implement suggested improvements to the EURISCO-EVA intranet | F. Guzzon/S. Kumar | 31/12/2023 |
| 5 | Provide genotyping experiment metadata for EURISCO-EVA database | H. Budahn | 31/12/2023 |
| 6 | Provide update on carrot virome project and results where possible | E. Geoffriau | 31/01/2024 |
| 7 | Create summary files with images to be uploaded as supplementary files to each experiment, where available | F. Guzzon | 31/01/2024 |
| 8 | Upload accession photos to EURISCO-EVA intranet | F. Guzzon | 31/01/2024 |
| 9 | Conduct data analyses according to assigned tasks | All assigned | 31/03/2024 |
| 10 | Propose accessions for regenerations by partners for potential new sets | Genebanks | 31/03/2024 |
| 11 | Process genotyping data for deposit in public molecular database | H. Budahn | 31/03/2024 |
| 12 | Propose model to correct trait data for density | M. Hardy | 31/03/2024 |
| 13 | Contact potential genebank, research and company partners to join EVA Carrot | S. Goritschnig | 31/03/2024 |
| 14 | Create Survey on priority traits for EVA Carrot 2.0 and collect input | S.Goritschnig | 31/03/2024 |
| 15 | Next meeting (online) | All | 31/03/2024 |
| 16 | Prepare standard protocol and background poster (with colour scale) for standardized picture-taking | tbd | tbd |