Summary report of the meeting

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

28-30 September 2022
Schagen and Warmenhuizen, The Netherlands

Contents

The 2022 Annual Meeting of the EVA Carrot Network was held in person in Schagen and Warmenhuizen, the Netherlands, co-hosted by Bejo Seeds. Partners who were not able to travel to the meeting connected online. The agenda of the meeting is attached as Appendix 1 and the list of participants as Appendix 2.

1. Welcome and introduction

Dorien Haarsma, representing Bejo Seeds, welcomed participants to the Netherlands and to the Bejo Open Days, which the participants were invited to during the meeting. This annual event allows Bejo's partners and customers as well as the general public to get a behind-the-scenes view of the company with organized tours of demonstration fields and the warehouse. Bejo is a family-run business with more than 1,000 employees worldwide, working on 40 crops, with a focus on carrots, onions and Brassicaceae. The EVA Coordinator, Sandra Goritschnig, opened the meeting, reviewing the agenda and reminding participants of the expected outcomes of the meeting.

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 8 countries, including 8 breeding companies.

Two EVA networks have already held in-person project meetings in 2022, and two more are planned for EVA Pepper in October and 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 project partners who presented their work during two international congresses in 2022. A news item has been published on the ECPGR homepage to highlight this EVA Carrot activity[1]. This project outreach has started discussions among other ECPGR crop working groups who are interested to initiate new EVA networks.

2. Results from EVA Carrot project

2.1 Review of network work plan 2019–2023

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 an SNP array. With all phenotypic data on this first set being finalized and uploaded to the

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[1] https://www.ecpgr.cgiar.org/resources/latest-news/news-detail/eva-carrot-network-results-shared-at-international-conferences-in-2022

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.

2.2 Review of selected accessions (criteria, previous knowledge, availability) and regeneration activities

Emmanuel Geoffriau (Institut Agro, France) reviewed the selection of accessions used in the EVA Carrot network so far. The goal had been to collect a representative diversity of carrots of European origin. The focus was on orange accessions of Nantes type, although some different types and colours were also included, especially from the Spanish collection which maintains interesting material. The material covers a range of geographic origins and should avoid having high tendency to bolt when growing in latitude over 42°. An important factor for selection was also seed availability, as all evaluations should use the same seed lot, thus requiring an available seed stock of around 30g.

Charlotte Allender (University of Warwick, UK) summarized the contribution from the UK Vegetable Genebank (UKVGB), which holds a large and geographically diverse carrot collection. Accessions were selected to fill geographic gaps in the collection. She highlighted that several accessions were also used in other projects, e.g. VeGin[2] and a carrot fly project. She provided an overview of the landraces and advanced cultivars that could be made available to the project for another set and noted that potentially interesting materials could be included in regenerations that are planned for 2022/23. Regenerations at UKVGB are usually conducted in glasshouses going from seed to seed. Annette Haegnefelt (Nordgen, Sweden) cautioned that using this approach and not regenerating with an intermediary root phase, the variety is not maintained. C. Allender confirmed that the goal of their regenerations is to maintain allelic diversity rather than the variety. Multiplication practices according to ECPGR recommend using around 100 plants for the regeneration of carrot populations to avoid genetic drift, thus using 50 plants is a compromise between technical possibility and the ideal case. For wild materials and landraces, more plants should be used where possible. In general, it would be better to regenerate fewer accessions with bigger populations, as this also would reduce the number of regenerations needed to maintain the material, reducing the risk of losing rare alleles.

A. Haegnefelt commented on the eight accessions provided by NordGen for the project, representing Nordic diversity from Sweden and Denmark. The accessions originated from breeding companies and some have been used to generate good hybrids. In their regenerations, Nordgen uses the root-to- seed approach because a new variety can appear within a generation. They typically use 60 roots for regeneration with 30 roots as a minimum for less fertile material.

Violeta Lopes (INIAV, Portugal) presented the small Portuguese *Daucus* collection, which is part of their MAP collection and contains mainly wild materials. The material was collected in Portugal 20 years ago and has never been regenerated. Twenty-two accessions were regenerated within the EVA project but at the moment only nine accessions have been successfully produced with more than 14g of seeds. Passport data is available for most accessions and especially the *D. carota sativus* collected in the wild had purple roots (with inner colour mostly yellow). Regenerations at INIAV are ideally conducted with 40 plants, using one cage per accession. The collection is old, and the quantity and quality of seeds are not uniform.

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[2] https://warwick.ac.uk/fac/sci/lifesci/research/vegin/

2.3 Preliminary results of carrot genotyping

Holger Budahn (JKI, Germany) presented the results from carrot genotyping, acknowledging the contribution of the JKI bioinformatics group around Heike Lehnert and Jens Keilwagen and also Master's student Tom Burges who conducted most of the work. Due to a drop in costs of GBS since first budgeting, the network was able to use different approaches to generate genotyping data: GBS on ten individual plants per accession as well as whole genome sequencing (WGS) and a new SNP array from TraitGenetics on one selected plant per accession, respectively. In addition to the 60 EVA accessions, 6 control accessions were genotyped, to create overlap with the CarrotDiverse project and to include the genome reference DH1. These additional accessions were also included in the phenotyping experiments at JKI, described by Thomas Nothnagel.

The number of informative SNPs was around 16k from GBS, 24k from the SNP array and over 7 million from WGS; this last dataset was reduced to facilitate analysis. In general, results from the three techniques are similar and it will be interesting to compare them in detail to assess the most useful and economical approach for the future. Roger's genetic distances were calculated for the accessions, which surprisingly mostly had values between 0.1 and 0.25, similar to cultivars, while this should be expected around 0.3 for landraces. Principal component analysis (PCA) showed that the material is genetically very diverse and that landraces from Spain clustered separately from the other accessions, indicating their uniqueness. It was suggested to analyze the data without the Spanish cluster to better separate the diversity of the remaining accessions.

Data analysis will be finalized by the Master's student, and the data will be prepared for upload to a molecular database. H. Budahn will provide relevant metadata for the genotyping experiments to be saved in the EURISCO-EVA database. The comparison of the different genotyping methods could be included in a scientific publication. GWAS could be done on the 660 plants from the GBS data, since phenotypic data is also available for the individual plants of the accessions from the trials conducted at JKI. The best markers could be developed into kompetitive allele specific PCR (KASP) markers for breeding and genebank management. TraitGenetics may consider developing a smaller array that could reduce the price for future genotyping.

JKI will interact with ECPGR to identify the most suitable public repository to host the genotyping data, with restricted access to partners during the embargo period. The possibility of implementing an SNP viewer for carrot will be investigated.

2.4 Preliminary results from evaluation trials

Evaluation partners presented results from their trials in 2022, including where possible also some initial statistical analysis.

E. Geoffriau noted that the trial in Angers in 2021 provided good results on powdery mildew, noting that plants infected by mildew turned brown later on, similar to leaf blight. Plant density affected trait scoring and some traits could not be scored on bolting plants. Brix degrees were measured on the samples and they showed high variation, but little GxE or year effect. On the other hand, foliar, root and disease traits showed significant year effects. He noted that similar to the genotypic data also in the analysis of the phenotypic data the Spanish accessions clustered separately. He suggested standardizing the representation of accessions in figures and publications, for example using an acronym including year, country of origin and EVA-ID, e.g.

1E28 (for year 1, Spain ESP, EVA_Dc_0028). This would facilitate the comparison of different analyses and the reuse of figures without having to redraw them.

Juliette Chevalier (Limagrain, France) presented results from their trials, noting that their trial in 2021 also gave good results in disease screenings. They did not observe much variation in leaf morphology which may not be useful descriptors. The trial had no root damage, enabling to observe the full potential of the accessions. Based on statistical analysis they detected significant year and genotype effects on their data, while replicates did not have a significant effect. The cause of the year effect is not clear, since the trials were grown in the same field under similar conditions. The carrots were grown on ridges, which helps to avoid flooding in autumn. It may be useful to include the different cultivation styles (ridges, raised beds etc.) in the metadata of the experiments.

A. Haegnefelt reported on her trial in 2021, for which they were collaborating with a farmer in southern Sweden and used a field where carrots had not been grown for a long time, resulting in the absence of disease pressure. It may be useful to rotate fields for carrot cultivation, to reduce disease pressure and crop protection input. She recorded seedling vigour by counting leaves and recording the average, a method which was proposed to the consortium to also apply for their scoring. She also highlighted the effect of plant density on the root size, so larger carrots are grown at lower sowing densities. She noted different average plant densities in the three beds used for the experiment and correlated root length and diameter.

Paolo Pagan (Carosem, Italy) noted that the growing season of the trials in the different locations resulted in different day lengths, which may affect bolting and other traits and would need to be taken into consideration.

Sylvia Salgon (Takii, France) noted that the two trials were conducted in different locations with a different trial setup and more space between rows in 2021, to facilitate scoring. Field trials were generally healthy with some powdery mildew observed at the end of the trial. Harvesting was difficult due to the heavy soil predominant in the south of France. Root cracking was common and often led to rotting. In the post-harvest evaluations, they observed some *Sclerotinia* infection and variation in general root quality. Since they did only evaluate one replicate, their statistical analysis focused on the comparison between the two years. Plant density was higher in 2020, which may be due to environmental effects or differences in germination. Root diameter was higher in 2021 when the plant density was lower, but root length showed no significant difference. They observed similar bolting behaviour in both years, except for one accession which only bolted in 2020.

Diana Katschnig (Bejo, the Netherlands) summarized the results from their disease trials. They had conducted powdery mildew field trials in 2020 and 2021, but both failed due to low infection or flooding, therefore a greenhouse trial was conducted in 2021. In this trial individual plants were infected with a high inoculum, resulting in most plants being infected. Six accessions showed segregating resistance to mildew. In the *Alternaria radicina* field trial 2020, they had first used the proposed EVA scale, however, afterwards decided to use their in-house scale which allowed a more quantitative and fine-grained differentiation. Most accessions were quite susceptible to the fungus but three accessions showed consistently fewer symptoms over the two years. The trials were conducted with one replicate only but the data were corrected for spatial effect and infection pressure using internal controls and are very robust.

Miguel Santillan Martinez (BASF Nunhems, the Netherlands) summarized results from the disease trials performed in their greenhouse. The trial for *Alternaria* and *Cercospora* leaf blight and cavity spot was conducted in a field pre-infected with different *Pythium* spp., and variation was observed in the response to those pathogens. Trials to root-knot nematode were conducted in pots in the greenhouse and all accessions were sensitive to *Meloidogyne incognita* while some showed intermediate resistance to *M. javanica*. He noted that the score which could be interesting for breeders to use depended on the trait, if there is no other resistance available, some intermediate resistance could be interesting. The trials included market standards, which should be used in comparison with the accessions. E. Geoffriau noted that *M. javanica* did not originate in Europe, so the accessions in the EVA collection did likely not coevolve with the pathogen.

Arnaud Thabuis (Rijk Zwaan, France) noted that they had performed root evaluation trials over two years in France and the Netherlands but focused his report on the flowering trial conducted in 2021. Accessions were sown in the fall of 2020, vernalized in a cold tunnel and transplanted to the open field in the spring of 2021. At the bolting stage plants were scored individually three times per week from the bolting stage till the harvest date for different flowering traits. Data recorded in the database are an average of ten plants. Several accessions had a significant percentage of male sterile plants. Most traits showed significant genotype effects. Trait scores showed a high correlation between the first and second umbel, although it is known that fertility can be different between different umbels. The accessions showed a higher level of branching compared to the included control variety. A. Haegnefelt recalled that the CarrotDiverse project trials had different seed yield in the two years, possibly due to differences in presence of pollinators. Considering the large amount of data collected in the flowering trial, partners discussed which would be the priority traits of interest. Plant structure and vigour as well as fertility and seed setting were considered most important, as they ultimately affect seed yield.

T. Nothnagel (JKI) provided a summary of the greenhouse trial conducted at JKI in 2020. Ten plants of each accession were evaluated for a number of morphological traits that had not been scored in the field trials and were also sampled for the GBS genotyping, possibly allowing GWAS analysis for some of the traits. Material was also collected and frozen for potential chemical analyses in 2023 if funding is identified. Most data were collected using picture-capturing technology. Trait score variation showed normal as well as bimodal distribution, which may affect data use in GWAS or QTL analyses. Partners should discuss which data are relevant, interesting for breeders and, most importantly, corresponding to field trials. One of the traits evaluated was cotyledon width and length. This was done as a follow-up to the CarrotDiverse project where large variation for these traits had been observed. It may be interesting to analyze the correlation between cotyledon traits and seedling vigour or seed weight. Only three accessions tended to bolt under greenhouse conditions. Root traits also showed good variation over the accessions. However, growing conditions should be taken into account when comparing with data from field trials.

In addition to morphological parameters, biotic stresses with different pathogens were also assessed on excised leaves and root discs of the same individual plants. Symptoms were recorded with a Lemnatec digital imaging system which allows quantitative analyses. It will be interesting to correlate these results with the natural field infections as well as the greenhouse disease trials of other partners.

T. Nothnagel questioned how the data should be curated for upload to the database, given that ten individual datapoints per accession had been recorded, whether each datapoint or a mean value should be recorded. In the long term and for upload to Eurisco recording an average value would be better, as the data should relate to the population and not individual plants. For in-depth analyses (including GWAS) it may be advisable to use the individual datapoints, especially given there are genotyping data available for each evaluated plant. Outliers should be recorded as in field trials, using the remarks field in the data collection template. For GWAS outliers may need to be disregarded. A first trait to be analyzed by GWAS will be the root type of the accessions observed during the experiment. The additional accessions included in the lab trials and genotyping will be assigned EVA-IDs.

Nicoletta Bertolin (Bejo, The Netherlands) noted that she expected planting density to affect root traits also in field trials.

3. Data management

3.1 Review of data collection templates

Based on the discussions during the presentation of trial results some suggestions were made to modify the data collection template and related traits metadata.

In order to better differentiate data collected on the same traits at different time points during the trial, which are currently differentiated only by the observation date, it was agreed to assign different trait-IDs to these traits.

The trait scores for natural disease infections in the field use a simple score to allow easy scoring during multiple time points. Specific disease assays performed by partners using their own disease scoring should consequently be defined with different trait-IDs and scoring scales.

The current scoring scale for seedling vigour is based on the BBCH scale, which may not be sufficiently discriminating. Partners suggested using seedling height as a measure, although this may also depend on the type of carrot. A. Haegnefelt proposed to measure energy levels as a proxy for vigour.

Partners discussed the best way to score heterogeneous materials and agreed to proceed with using the score 'other' to indicate segregating traits and the remarks field for detailed description of the observation. Additional remarks for accessions that should be added in the metadata include colour, shape, growing cycle (early/late) and uniformity.

3.2 Hands-on session with EURISCO-EVA intranet

Suman Kumar (IPK Gatersleben), developer of the EURISCO-EVA intranet led a hands-on session of the database storing phenotypic data of EVA trials. Partners can upload data directly to the platform, using the standard data collection templates developed for this purpose. Several check steps are implemented during the upload ensuring the validity of the data, e.g. comparison of allowed values for trial-ID, trait-ID and trait scores. Upload audit logs provide feedback to users and a guide was developed to assist in the use of the platform. User documents that can be downloaded from the intranet include the data collection templates and other user guides under development. The database holds relevant metadata for the network, including passport data of

carrot accessions, information on experimental details and descriptions of traits evaluated. Up to five photos can be uploaded per accession as associated metadata which can be linked to a certain trait or experiment, where suitable. Another feature is the possibility to attach supplemental data files to experiments, such as field plans, weather data or initial statistical analysis, which will not be processed and enter the database but can be used for reference. The database is not designed to hold genotyping data but will store metadata for genotyping experiments and links to data repositories. S. Kumar demonstrated the various filters and search methods available for analyzing the datasets as well as display functions. While the database does not offer functionality for statistical analysis, users can download search results and filtered data and metadata for in-depth analysis.

Participants commended the development of a useful tool to enable the analysis of standardized data. Data from most experiments of the EVA Carrot network have already been uploaded, adding up to more than 80,000 individual data points from 26 trials. Some suggestions were provided to improve the visual display of the database.

It was suggested to be able to display trait score distribution for different trials and years to visualize potential year effects. Comparing the ranking of accessions based on trait score in different trials could identify traits with significant genetic or environmental effects.

EVA Carrot partners collected many carrot photos during the experiments. It was suggested to combine several images from one trial into one file to allow the upload of images from multiple locations (e.g. Sweden, Netherlands, France, Spain and Italy). It was further suggested to display information on trial location and year with each photo. In order to select the most suitable and representative images for each accession, a collage of photos from the different field trials will be generated. Partners will then jointly decide on the photosets to be uploaded to the database. Additional photos can be combined into one pdf file and uploaded to the relevant trials as supplemental data.

S. Goritschnig confirmed that the data collected in the EURISCO-EVA intranet could be easily transferred to the public EURISCO database after the expiration of the embargo period and upon approval of the national coordinator of the collection holding a certain accession. Given the intention of ECPGR to further promote the EVA concept and continue supporting the EVA networks, the database will be maintained and improved where possible as long as EURISCO is part of ECPGR.

4. Data analysis

Discussions on data analysis approaches were started with a brainstorming exercise where each partner was asked to respond to the following questions: 1) What are my main objectives to get from the data (research/breeding)? 2) Which specific questions would we like to answer? 3) How should we analyze the data (methods/approach)?

Partners agreed that disease resistances were priority traits of interest. While breeders' main interest was in identifying material and perhaps associated QTLs or genetic markers, further in-depth research into the genetic basis and mechanism of disease resistance were interesting but considered beyond the scope of the EVA network. All partners were interested in investigating the genetic diversity of the accessions and identifying genetic determinants for different traits.

Another important focus was defined as comparing the variability of accessions for different traits in different locations in order to correlate genetic and environmental effects and identify the most discriminatory traits and locations for future trials. Similarly, the description of a core set of traits (priority descriptors) or accessions (core collection) for a certain research topic was considered desirable. Analysis of the potential links between traits and the origin of accessions would be interesting. Breeders were also interested in morphological characterization data to identify material that could enrich their breeding germplasm for different traits. Genebank curators had an interest in observing their materials in different locations and the potential to improve their collection management based on diversity studies. Performing correlations between the results from lab, greenhouse and field trials for specific traits was also considered interesting. C. Allender further suggested integrating the EVA Carrot data with datasets from previous studies (e.g. CarrotDiverse) to compare the scale of genetic diversity to the carrot genepool as a whole. A. Hagnefelt would use the data to improve the description of accessions in the genebank database, as at the moment very limited data is available. This could at first be implemented by including short descriptions in the remarks field of each accessions.

Specific questions that partners were interested in addressing included investigating the evolutionary origin of the differentiation of the Spanish landraces, the genetic and environmental effects on traits, correlations between phenotyping and genotyping data including QTL and GWAS where possible, correlations between lab and field trial data, identification of environmental factors most influential for carrot development, determining the relative importance of year and location effects, identifying a proxy trait for seed set, assessing whether certain traits could be predicted from early observations (e.g. bolting from seedling vigour). While the number of accessions in the field trials may be too low for association studies, the diversity of trial locations was considered an asset. A comparison of the genotyping methods in terms of data quality, quantity and cost could be useful for further studies as well as genebank curation.

In terms of analysis approaches partners suggested conducting classical variance analysis for statistics, as well as PCA to identify the contributions of genetic and environmental factors on quantitative traits. rPLS is a method using multiple regression to allow the identification of a hierarchy of the most influential factors on certain traits. A definition of new criteria, such as ratios or functions of the collected raw data, may also be useful to better describe certain traits. Taking advantage of image analysis may be suitable for the future, but will require standardized picture-capturing tools such as Lemnatec. Where possible, GWAS should be conducted on disease traits as well as flowering and quality traits. Gathering as complete as possible metadata on geographical location, soil and weather conditions, was highlighted as an important step.

C. Allender mentioned an ongoing project by John Clarkson at the U. of Warwick, who is developing a useful protocol to assay cavity spot and suggested that the EVA network could share information and knowledge. If available, seeds of the EVA accessions could be shared with J. Clarkson and included in trials.

Based on the input from the brainstorming and considering the expertise and main interests of partners, a working document containing a task list was developed that divided the data analysis into different topics and approaches and assigned them to partners with target deadlines in 2023. Tasks included general questions such as analysis of variation and genetic vs. environmental effects, breeding-related questions such as analysis of disease resistance traits and relevant GWAS as well as evolutionary genetics studies looking at the general diversity of the collection.

It was also clarified that during analyses partners would have access to all datasets generated in the network. In addition, topics that could be part of an EVA carrot 2.0 project were identified and assigned to partners to be further developed in a project plan.

5. Outlook

5.1 Dissemination and exploitation of results

The EVA Carrot network presented preliminary results during international meetings in August 2022. P. Pagan (Carosem) gave a talk on behalf of the consortium during the International Horticultural Congress in Angers, France (https://www.ihc2022.org/) and a paper was prepared and submitted to the congress proceedings for publication in *Acta Horticulturae*. A. Thabuis (Rijk Zwaan), together with other network partners presented a poster at the International Carrot Conference in Mt. Vernon, USA (http://www.internationalcarrots.org). A news item on these activities was published on the ECPGR and EVA websites[3]. Disseminating initial results of the network in this way provides visibility to the project and is an important activity for partners to report to their management on the progress made.

Partners were invited to attend the III International Symposium on Carrot and Other Apiaceae in York, UK from 2-5 October 2023 (https://www.carrotsymposium.com/) to present the various results of the network. An EVA Carrot project meeting could be organized on the sidelines to discuss future collaborations, preferably on Monday, 2 October.

Given the large amount of data generated in the different experiments, several publications may be developed on different topics. Partners discussed what data and in which way could be incorporated in publications, taking into account the priority traits of the breeders and embargo period. For example, multivariable analysis of data could be presented in global diversity studies without explicitly identifying the source for a specific trait. Breeders identified disease resistances and associated accessions as sensitive data that should not be used for publication during the embargo period. The data from the flowering trial should also be considered sensitive. Partners agreed that all data can be used in global analyses as long as genotypes cannot be associated with a specific trait. It was emphasized that the accessions are genebank materials and thus, in theory, publicly available. The genotyping data will not be embargoed but their release in public databases will necessarily be implemented coinciding with any publications generated.

5.2 Proposals for EVA Carrot 2.0 – continuation of network activities

S. Goritschnig reminded partners that the EVA project's intention is that EVA crop networks continue in a self-sustaining way after the end of the current funding period. ECPGR is currently developing a work programme for its XIth Phase (2024−2028), in which the EVA project may play an important role and could receive funds from the regular budget. However, project-specific activities (e.g. genotyping, evaluations, data analysis) would still need to be covered by external funds or in-kind contributions. Furthermore, the involvement of the ECPGR Working Groups (WG) could be improved and new genebank members added to the network. At the moment, the

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[3] https://www.ecpgr.cgiar.org/resources/latest-news/news-detail/eva-carrot-network-results-shared-at-international-conferences-in-2022

EURISCO database contained 4,370 carrot accessions, but none with associated phenotypic data, so there is still a large potential genepool available to be tapped for breeding.

E. Geoffriau recalled the set of minimum descriptors developed by the Umbellifer WG for cultivated and wild carrot accessions, noting that its use is not well implemented by genebanks. These traits were included in the network experimental protocol.

Partners discussed issues to be considered for future collaboration.

D. Haarsma noted that the 66 accessions used in this first round of the EVA project were a good start and Bejo was interested in continuing the collaboration. Ideally, more accessions should be used in the trials, perhaps evaluated with fewer traits, with a focus on disease resistances. It would also be interesting to evaluate more wild relatives, as they may provide new resistance alleles. A. Hagnefelt suggested that disease trials could focus on the leaves as that is where the diseases start, thus reducing the number of plants needed. One good trial could be sufficient to assess disease resistance.

E. Geoffriau suggested that the data collected so far will allow for assessing how many trials in how many locations would be necessary or sufficient to assess different traits, including resistances, across accessions. Based on this, a future work plan could better distribute the work among the network partners.

Bejo and BASF offered again to contribute to regeneration activities, keeping in mind that their standard procedure would need to suit the genebanks.

Public partners such as NordGen, Institute Agro and CITA depended on funding to be able to conduct field trials. Genotyping was also considered an important activity that needed a budget to generate data useful for breeding activities, and data analysis was emphasized as a time and resource-intensive activity that should be considered in future project planning and budgeting. Other additional traits addressing organoleptic and quality traits would also require funding sources.

5.3   General discussion and next steps

Given the current project end date of November 2023 as well as the plan to present results during the Carrot Symposium in October 2023, data analysis activities should be scheduled to be finalized by summer 2023. Partners were reminded to use standard methods and consider a standard annotation of accessions in figures so they could be used across publications.

One target outcome of the data analysis could be a prediction for the best combination of three trials for future phenotyping of specific traits. Future work planning could also split trial sets such that quantitative traits with significant year effects could be assessed on a larger set of accessions in the first year, from which a subset could be selected for in-depth evaluation. Selection could be assisted by genotyping data. Evaluation of qualitative traits such as the minimum descriptors should be continued but could be sufficient from one year in fewer locations. Planning of future experiments will depend on the results from the analyses conducted in 2023.

It was suggested to conduct germination tests for the next set of selected accessions before sending the seeds. The total amount of seeds needed for future trials will depend on the experiments and the number of trials to be planned.

One partner questioned how to control for development time of accessions (early vs. late), and suggested taking into account the carrot type which may affect development due to a cycle effect, to be included as a co-depended variable.

Partners were reminded to provide weather data for their trials, if possible from a dedicated weather station (recording temperature, light intensity and rainfall) as well as soil characteristics of the trial site and any specific cultivation practices if available. National weather data could also be collected to provide a general and standardized overview of the weather pattern at different locations. These will be uploaded as supplemental data to the individual trials in the EURISCO-EVA database and will be important to assess the environmental effects.

E. Geoffriau recalled the carrot virome project, for which partners had provided leaf material from their trials. Unfortunately, the samples from NordGen were destroyed during shipping and were not included in the project. He will inquire about the status of the project and results and report during the next meeting.

Partners agreed to continue work on a pre-competitive level, focusing on the evaluation of landraces and perhaps also wild carrot accessions. A discussion opened on whether to expand the material to accessions with origins from outside of Europe (as long as they are available in EURISCO), but it was considered to be more important to first gather more information on material from European origin, and to valorize those, feeding into core collections and the AEGIS collection. It was agreed to see how big a set could be managed by the project and how this should be made up. In general, wild carrots of European origin should be prioritized over international accessions.

Other genebanks with significant and diverse carrot collections could be invited to participate in the network, such as those in Poland, the Czech Republic and Germany. This could happen through involving the ECPGR Umbellifer WG and should also take into account necessary regeneration activities. If regenerations should be conducted in 2023, new material should be identified by June 2023.

Next steps to be organized (see action list in Appendix 3) include finalizing data curation by November 2022 to enable data analysis by partners. Initial results would be shared in a virtual meeting in spring 2023, discussing their further development into publications. An in-person project meeting in October 2023 could be used to review the presentations and papers coming out of the analysis and to develop the project plan for EVA Carrot 2.0, based on a survey shared among project partners in spring 2023 and potential funding options. This survey would list possible topics and approaches and allow partners to indicate their priorities.

6. Close of the meeting

S. Goritschnig thanked participants of the meeting for their active participation and acknowledged the efficient strategic discussions during the in-person meeting that enabled significant progress to be made. She also thanked Bejo for co-hosting the meeting and the informative visits to the warehouses, molecular lab and fields.

The next virtual meeting, to review initial data analysis results, will be scheduled in spring 2023, on a date to be determined.

Appendix 1. Meeting agenda

Venue: Golf club Dirkshorn

WEDNESDAY 28 SEPTEMBER		
13:15 – 14:00	Registration and transfer from Hotel Marktstad, Schagen to venue	
	Welcome and introductory session	CHAIR: S. GORITSCHNIG
14.00 – 14.10	Welcome by Bejo, ECPGR	*D. Haarsma, S. Goritschnig*
14.10 – 14.20	Introduction of participants	*All*
14.20 – 14.30	Overview of the current status of the ECPGR Evaluation Network EVA	*S. Goritschnig*
	Results from EVA Carrot project	CHAIR: E. GEOFFRIAU
14.30 – 14.40	Review of network workplan 2019-2023	*S. Goritschnig*
14:40 – 15:00	Review of selected accessions (criteria, previous knowledge, availability) and regeneration activities	*E. Geoffriau A. Hagnefelt C. Allender V. Lopes*
15:00 – 15:30	Preliminary results of Carrot genotyping	*H. Budahn*
15.30 – 15:50	**TEA/COFFEE BREAK**	
15:50 – 17:00	Preliminary results from evaluation trials (~5-7 mins each) Field trials Flowering trial Lab trials Disease trials	*S. Salgon A. Haegnefelt J. Chevalier E. Geoffriau P. Pagan* *A. Thabuis (flowering)* *T. Nothnagel (lab trials)* *Miguel Santillan Martinez (nematode)* *Nicoletta Bertolin (Alternaria and mildew)*
17:00 – 17:30	Discussion	*All*
18.00	**SOCIAL DINNER AT GOLF CLUB DIRSKHORN**	

Venue: Bejo Seeds, Warmenhuizen

THURSDAY 29 SEPTEMBER		
8:30	Transfer from Hotel Marktstad, Schagen to venue	
	Data management	**S. GORITSCHNIG**
09.00 – 09.20	Review of data collection templates	*S. Goritschnig*
09.20 – 10.20	Hands-on session with EURISCO-EVA intranet	*S.Kumar remotely*
10.20 – 10.40	**TEA/COFFEE BREAK**	
	Data analysis	**CHAIR: E. GEOFFRIAU**
10.40 – 11.40	Brainstorming and discussion on data management and analysis – possible questions, approaches, volunteers	*All*
11.40 – 12.30	Planning of data analysis work pipelines for 2022/23	*All*
12:30 – 13.30	**LUNCH**	
13.30 – 15:30	Bejo open day. Visit to seed processing warehouse	*D. Haarsma*
15.30 – 16.00	**TEA/COFFEE BREAK**	
16.00 – 17.00	Bejo open day. Visit to the carrot field	*N. Bertolin*
17:00	**TRANSFER TO SCHAGEN AND FREE EVENING**	

Venue: Bejo Seeds, Warmenhuizen

FRIDAY 30 SEPTEMBER		
8:30	Transfer from Hotel Marktstad, Schagen to venue	
	Outlook – EVA Carrot 2.0	**CHAIR: E. GEOFFRIAU**
09.00 – 10.00	Dissemination and exploitation of results Reports from international meetings • IHC Angers • ICC Mt Vernon	*S.Goritschnig* *E. Geoffriau/P. Pagan* *A. Thabuis*
10.00 – 10.30	Proposals for EVA Carrot 2.0 – continuation of network activities	*E. Geoffriau,* *S. Goritschnig*
10.30 – 11.00	**TEA/COFFEE BREAK**	
11.00 – 12.00	Discussion on continuation of network activities after end of current project (Nov. 2023)	*All*
12:00 – 12.30	Wrap-up of meeting	*S. Goritschnig*
12.30 – 13:30	**LUNCH**	
13:30	End of meeting and Transfer to Schagen	

Appendix 2. List of participants

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Appendix 3: Action list

Number	Action	Responsible	Due by
1	Curate datasets according to agreements during meeting (new trait-IDs, updated controls)	S. Goritschnig	30/11/2022
2	Update accession set with info from JKI extra accessions	S. Goritschnig	30/11/2022
3	Update experimental protocol, data collection template and standard protocols based on discussions from meeting	S. Goritschnig	15/12/2022
4	Implement suggested improvements to the EURISCO-EVA intranet	S. Goritschnig/S. Kumar	31/12/2022
5	Provide genotyping experiment metadata for EURISCO-EVA database	H. Budahn	31/12/2022
6	Provide relevant additional metadata for field trials (weather data, cultivation style etc) and upload to EURISCO-EVA trials	All/S. Goritschnig	31/12/2022
7	Submit abstracts to Carrot Symposium by deadline	All	30/01/2023
8	Provide update on carrot virome project and results where possible	E. Geoffriau	31/01/2023
9	Propose accessions for regenerations by partners for potential new sets	Genebanks	31/03/2023
10	Conduct preliminary analyses according to assigned tasks	All assigned	31/03/2023
11	Process genotying data for deposit in public molecular database	H. Budahn	31/03/2023
12	Create collage of available images of accessions to select the most suitable ones for upload to the database as metadata	S. Goritschnig	31/03/2023
13	Collect short standard descriptions of accessions to be included in the remarks field/metadata	S. Goritschnig/A. Haegnefelt/E. Geoffriau	31/03/2023
14	Next meeting (online)	All	01/04/2023
15	Create survey for project planning on EVA carrot 2.0 after spring meeting	S.Goritschnig	30/04/2023
16	Create summary files with images to be uploaded as supplementary files to each experiment, where available	All	30/06/2023
17	In-person project meeting at international carrot symposium	All	02/10/2023