



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



20 April 2021
online MS Teams

Summary report of the meeting

Contents

1. Welcome and introduction.....	2
2. Review of project progress – trials 2020.....	2
2.1 Review of project proposal and general update	2
2.2 Updates from 2020 trials	2
2.3 Preview of 2021 trials and general discussion	4
3. Outlook for 2021	5
3.1 Review of multiplication activities – Regeneration of accessions	5
3.2 Genotyping: objectives and selection of technology and provider	5
3.3 Data analysis – methods and responsibilities	6
4. Close of the meeting.....	7
Appendix 1. Meeting agenda.....	8
Appendix 2. List of participants.....	9
Appendix 3: Action list	11

The 2021 Annual meeting of the EVA Carrot Network was convened online on 20 April 2021, 13:30 to 17:00, on MS Teams. 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, reviewing the agenda and reminding participants of the expected outcomes of the meeting and the functions of the virtual meeting platform used. She voiced the hope that the next annual meeting in 2022 would be held in person.

2. Review of project progress – trials 2020

2.1 Review of project proposal and general update

The EVA Coordinator informed partners on latest developments affecting the EVA carrot project. Owing to delays experienced by partners in multiple networks due to the Covid-19 pandemic, the ECPGR Secretariat had requested a no-cost project extension until November 2023 from the donor, which was granted. This will allow delayed project activities to be completed and, hopefully, provide an opportunity to organize in-person meetings, important for strategic discussions related to project continuation. Within the EVA Carrot Network specifically, this extension could be used to plan evaluations of a new set of accessions in 2022.

Network partners were informed that the cooperation agreement with all signatures was uploaded to the project sharepoint. Charlotte Allender (University of Warwick, UK) apologized for the delay in securing her institution's signature and assured that it would be provided as soon as possible.

The EVA intranet was under development, having faced a delay because of the Covid-19 pandemic. Suman Kumar (IPK Gatersleben) was working on the platform which will be used to collect and store the phenotyping information and link with EURISCO. A prototype will be shared with partners for feedback later this year.

The EVA webpage has been updated and now includes additional information: <https://www.ecpgr.cgiar.org/european-evaluation-network-eva/eva-networks/carrot>.

Partners were invited to review it and provide feedback to the EVA Coordinator.

Partners were reminded of the overall workplan whose activities include evaluating 60 accessions for agronomic and biotic stress traits in laboratory and field trials, regenerating gene bank accessions to ensure availability in collections, genotyping and joint data analyses. A number of open questions on the workplan were discussed during the meeting.

2.2 Updates from 2020 trials

Evaluation partners provided brief updates on their trials in 2020, focusing on highlighting interesting results, challenges faced and suggestions for improvements for the replicate trials scheduled for 2021. Evaluators were reminded to upload their data using the data collection templates to the project sharepoint, for detailed analyses.

Thomas Nothnagel (Julius Kühn-Institute (JKI), Quedlinburg, Germany) recalled previous updates on the laboratory trials which had been presented to partners. All data for characterization and disease tests were ready and would be uploaded to the sharepoint. He noted the collaboration with the Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection in Berlin, who are currently performing chemical analysis for polyacetylenes and volatile compounds for material collected during the trials. JKI was also leading the coordination of the genotyping activity and an overview of possible approaches was provided. Detailed discussion of this topic is described later in this report.

Arnaud Thabuis (Rijk Zwaan, France) reported on their two trials in the Netherlands and France. He noted that due to the Covid-19 pandemic, sowing had been delayed resulting in different disease pressure than under regular conditions. In the trials they observed leaf blight and powdery mildew infections which, in their trial in France, was not evenly distributed across the field. Colour variation in the roots was less pronounced in the Dutch trial. The flowering trial was ongoing and seedlings had been transplanted in the field in March.

Paolo Pagan (Carosem, Germany/Italy) reported on their trial in Northern Italy. The trial site had experienced unusually cold and dry weather, especially in October to November of 2020, which affected the disease pressure for this trial. He expressed a need for better picture guidance especially for disease scoring to facilitate evaluations and noted that he would need additional seed for some accessions to repeat the experiments. Brix measurements were only done after 2,5 months of storage, due to personnel shortage, so these may not be comparable with results from measurements taken at harvest.

Cristina Mallor (CITA Aragon, Spain) reported that they had applied some herbicide treatment during sowing for weed control and also sampled the soil at harvest to record its composition. She noted some difficulties with the storage trial, as the roots were greatly desiccated after two months.

Micha Groenewegen (Sementes Vivas, Portugal) noted that due to Covid-19, they had to change the location for the trial to the Algarve, which also delayed the sowing to late in October. He informed that the plot used for the trial was certified organic land but that the trial was difficult to manage from a distance. Harvest occurred in mid-March, evaluation data were being summarized and the storage trial was still ongoing. He noted poor germination for some accessions and some difficulty scoring segregating traits and colour variation.

Sylvia Salgon (Takii Europe, France) reported that her trial in Avignon included only one replicate, but used carrot borders to reduce a border effect and several internal controls. She noted a number of early bolting accessions, as well as some overgrowing effects which may have affected several genotypes. She also observed segregation for root colour and frequent root cracking at harvest as well as some Sclerotinia symptoms in the storage trial.

Juliette Chevalier (Vilmorin & Cie, France) noted that in their trial, the germination was generally good and results for disease traits were interesting. She noted that there was little variation in some foliar traits and questioned whether another scoring for these was necessary.

Aurélie Ingremeau (OBS, France) reported that in their trial, they had a lower plant density than recommended in the experimental protocol, which may have been due to problems during hand sowing. She noted, however, that results for disease traits were interesting and most characterization traits could be scored. Due to rain in the fall, the foliage displayed more yellowish colouring.

Nicoletta Bertolin (Bejo Zaden B.V , the Netherlands) noted that, since Bejo had only joined the network in May 2020, the trials were sown late and had lower germination. They had performed two controlled disease trials in the field, but the one for powdery mildew did not produce reliable results. She noted that the trial for *Alternaria radicina* had shown good results and proposed to use a more fine-grained rating system for scoring this trait.

Emmanuel Geoffriau (ACO-IRHS, France) reported that their trial had shown good disease pressure for powdery mildew and leaf blight and that they had noted a latitude effect for bolting of accessions from the Mediterranean. He also performed Brix measurements and observed interesting variation among accessions. He commented that in the trait descriptions, it should be clear what timepoints were scored (e.g. roots at harvest versus post harvest) and the duration of the harvest needed to be taken into account if it takes several days.

2.3 Preview of 2021 trials and general discussion

Based on the reports on the 2020 trials, some adjustments to the experimental protocol, data collection templates and trait descriptions will be implemented.

Partners were reminded that pesticide treatments could be applied as last year but should be recorded in the data collection template. Ratings should be scored on both replicates where applicable.

The sowing dates of trials would be in line with those from last year and partners commented on their need to receive additional seeds for some accessions. This need will be communicated to E. Geoffriau, who will again centralize seed distribution and communicate with providing gene banks where necessary. Partners who needed additional seeds (or had not yet received seeds for their trials) were Carosem, Bejo and Nordgen.

Partners discussed modifying the traits list to reflect comments received. For example, E. Geoffriau suggested to score leaf colour intensity which had shown more variation instead of leaf colour per se and asked partners if this trait would be of interest to them. Annette Haegnefelt (Nordgen, Sweden) noted that leaf colour could be relevant for early maturing varieties which usually had more yellow leaves. Partners agreed to drop leaf colour and type from the joint evaluation. Leaf growth habit, on the other hand, was considered an important trait as it affects mechanical harvesting; however, the scoring scale suggested in the IPGRI descriptors was not considered detailed enough and should be modified to include intermediate scores and pictorial guidance included in the protocol if possible.

Partners were requested to provide feedback on traits where the scoring scale should be revised and also to provide pictures that could be used as guidance in the protocol.

E. Geoffriau recommended that the protocol for the storage trials needed to be revised to provide sufficient guidance. For example, roots should be sorted and only healthy material included in the storage trials, and high humidity should be ensured to avoid desiccation of material during storage.

E. Geoffriau reported on a research proposal of a partner in France who would like to evaluate the “virome” of carrot from trials across Europe. He explained that this would involve sequencing bulk leaf samples from different locations and identifying different viruses associated with carrot in different trials, and also to distinguish viruses associated with seeds from those infecting during cultivation. Partners agreed to share leaf material collected in their trials for this research project. More details would be communicated when they became available.

3. Outlook for 2021

E. Geoffriau led the discussion on activities beyond the field trials, highlighting the overall goal of the EVA Carrot Network to ensure availability of material at gene banks, and to increase evaluation data of EURISCO accessions from different environments.

3.1 Review of multiplication activities – Regeneration of accessions

S. Goritschnig reminded partners of a file on the project sharepoint, where regeneration needs and activities were listed, and asked partners to update this file as necessary.

A. Haegnefelt noted that several of the accessions provided by NordGen had limited seed amounts left and noted that they were not able to plan regenerations, inviting partners to step in. Charlotte Allender (University of Warwick, UK) noted that up to six accessions provided by the UKVGB need to be regenerated, but these could be done by their institute.

P.A. Dekker (Bejo Zaden B.V, NL) said that Bejo could carry out regenerations for the project of between 10-20 accessions, using 28 plants per cage. E. Geoffriau noted that it would be better to maintain the diversity in populations to use more plants per regeneration, a minimum of 56 was recommended.

S. Goritschnig invited partners to consider selecting a new set of accessions for a second round of evaluations, which was now possible in the extended project. These could be regenerated in 2021.

3.2 Genotyping: objectives and selection of technology and provider

During his update, T. Nothnagel presented several options for genotyping methods and providers which could be used in the project. Genotyping-by-Sequencing (GBS) would be the preferred option, as it was also used in the CarrotDiverse project (<https://www.ecpgr.cgiar.org/working-groups/umbellifer-crops/carrotdiverse>), is cheap and would allow the sequencing of up to 10 individual plants per accession, thereby allowing a more effective capturing of the diversity in each accession. A SNP array was presented as an alternative and JKI could get special pricing, but it would still be more expensive per sample. Another agreement with IPK Gatersleben would allow a combination of GBS and limited whole genome sequencing (WGS) within the allocated budget. Finally, JKI could lead the data analyses, as they had done also for CarrotDiverse.

Partners discussed the various approaches and their advantages and disadvantages. It was noted that GBS would require extensive bioinformatics analysis and data from different GBS experiments were not easily integrated as datasets depended on the protocol used for library preparation, especially for a crop with a complex genome such as carrot. The array could produce more reproducible results. However, since the array was designed based on cultivated carrots, it may not perform as well on wild accessions. WGS was considered a useful addition to be done on a subset of plants based on primary GBS or phenotypic data.

One partner noted that if phenotyping was done on plots, while genotyping was done on individual plants, the data may not be easy to correlate, especially since the material for genotyping had been collected from different plants that were phenotyped. Therefore, it was suggested to include a bulk sample (of up to 48 plants per accession) in the genotyping, in addition to the individual samples. It was highlighted that the read depth of the GBS should be considered in deciding the number of plants in the bulk sample (e.g. a read depth of ~20 would only provide one read on average per plant if 20 plants were bulked, or 0,5 if 40 plants were bulked).

One partner noted that it would be important to also define the goals of the genotyping, as GWAS and diversity studies would use different approaches in the data analyses. It was suggested to review the GBS protocol of CarrotDiverse and to use the same provider to increase the percentage of mergeable results of the two projects, even though expectations should not be too high for this.

Partners agreed to follow an approach combining genotyping of individual plants and bulks of accessions (e.g. 9 plants + 1 bulk), with the size of the bulk to be determined. Quotations from GBS providers would be requested, also keeping in mind the possibility to reserve some funds for WGS.

3.3 Data analysis – methods and responsibilities

Partners noted the need to revise and update the data collection templates, based on experiences shared and suggestions presented during the meeting. Further to the already discussed suggestions, these included identifying the data collected at different timepoints to facilitate time course analyses, differentiate root traits at harvest and post-harvest and to include the date of harvest, since this often occurred over multiple days.

Partners discussed different objectives of data analyses and how to ensure the data collections enable these. One partner noted that bolting was dependent on day length, but also correlated with temperatures and therefore climatic data should be collected for all trials to allow correlations with traits. Other interesting analyses were disease kinetics including GWAS or seedling establishment, which could be scored early in the trial as germination, vigour and seedling stage (based on BBCH) at the different timepoints.

Partners supported E. Geoffriau's proposal to valorise the generated results in publications as much as possible, keeping in mind the embargo periods for traits as necessary and ensuring that publications were circulated within the network for approval before submission. Partners were reminded of the classification of traits as within or without the embargo, which was agreed in the previous annual meeting and was noted in the cooperation agreement appendix.

4. Close of the meeting

S. Goritschnig thanked participants of the meeting for their active participation and briefly summarized next steps (see action list in Appendix 4). Partners were asked to provide their feedback on the meeting in an anonymous survey, in which all respondents subsequently expressed a general appreciation with the progress of the network and the outcomes of the meeting. Some open questions remained to be addressed and further discussed, such as the optimal experimental setup for the GBS genotyping to allow GWAS analysis, or how partners could access genetic material for future breeding activities. Some additional discussion about experiences during the field trials would be welcome to exchange suggestions for optimization, and data analyses approaches would also need further discussion among partners.

The next virtual meeting, to receive updates on genotyping and phenotyping activities, would be scheduled in November 2021, on a date to be determined.

Appendix 1. Meeting agenda

20 April, 13:30 – 17:00 (Venue: MS Teams)

13:15 – 13:30	Meeting room opened; technical assistance if needed	
	Welcome	
13:30 – 13:35	Welcome and review of platform and available files/tools	S. Goritschnig
	Review of project progress – trials 2020	Chair: S. Goritschnig
13:35 – 13:40	Review of project proposal and general update	S. Goritschnig
13:40 – 14:30	Updates from 2020 trials: (5 mins per presentation) <ul style="list-style-type: none"> - JKI - Rijk Zwaan - Carosem - CITA - Sementes Vivas - Takii France - Vilmorin - OBS - ACO/IRHS - Bejo 	<ul style="list-style-type: none"> - T. Nothnagel - A. Thabuis - P. Pagan - C. Mallor - M. Groenewegen - S. Salgon - J. Chevalier - A. Ingremeau - E. Geoffriau - P. A Dekker
14:30 – 14:45	Preview of 2021 trials	All
14:45 – 15:30	General discussion	
15:30 – 15:45	Break	
	Outlook for 2021	Chair: E. Geoffriau
15:45 – 16:00	Review of multiplication activities – Regeneration of accessions	All
16:00 – 16:30	Genotyping: objectives and selection of technology and provider	All
16:30 – 16:55	Data analysis – methods and responsibilities	All
16:55 – 17:00	Any other business	All
17:00	Close of meeting: Next meeting (as necessary): date tbd	S. Goritschnig

Appendix 2. List of participants

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Appendix 3: Action list

Number	Action	Responsible	Due by
1	Inform E. Geoffriau about need for extra seeds for relevant accessions	Partners	15.05.2021
2	Request seeds from gene banks (where needed) and distribute to partners	E. Geoffriau	31.05.2021
3	Collect feedback from partners on potential revisions to scoring scale for certain traits	S. Goritschnig	31.05.2021
4	Provide photos of traits to facilitate scoring	All	31.05.2021
5	Update experimental protocol, data collection template and standard protocols based on discussions from meeting	S. Goritschnig	30.06.2021
6	Provide data from first year trials on project sharepoint	All	ASAP
7	Request quotations from genotyping providers for agreed approach	T. Nothnagel and S. Goritschnig	
8	Generate bulk sample for genotyping	TBD	TBD
9	Provide additional information on carrot virome project and expectations from partners	E. Geoffriau	TBD
10	Select accessions for regenerations by partners from genebank stocks	All	ASAP
11	Next meeting (online)	All	Nov.2021