

Meeting Report of the ECPGR Activity "CarrotDiverse"



4-5 July 2017, Braga, Portugal

Meeting hosted by the Banco Português de Germoplasma Vegetal (BPGV), Braga

In attendance:

Charlotte Allender Thomas Nothnagel Emmanuel Geoffriau Violeta Lopes Ulrike Lohwasser Ana Maria Barata (observer) Ana Maria Carvalho (observer) Filomena Rocha (observer) Maria do Carmo Serrano (observer) Madalena Vaz (observer)

Meeting Summary

The first meeting of the CarrotDiverse project covered two broad areas; discussion and agreement of project protocols (traits for characterization and sampling strategies, timelines) and the European and global landscape of the project in the context of other significant collections and activities on wild *Daucus* species.

The project has two separate characterization activities. The first is a detailed comparison of 10 common accessions across three sites (Portugal, France, Sweden) along a latitudinal gradient, and the second is a more general characterization of approximately 30 different accessions at each site.

More specific evaluation activities were planned involving genotyping by sequencing of 96 accessions, and testing these accessions for resistance to *Alternaria*.

The context of the project in terms of global requirements for the conservation of *Daucus* species was discussed as well as previous and ongoing work in the Crop Wild Relatives project led by The Crop Trust and Royal Botanic Gardens, Kew.

Day 1 – 4th July 2017

Introduction and welcome

The meeting opened with a welcome and introduction from Ana Maria Barata on behalf of INIAV as hosting institute and as ECPGR National Coordinator for Portugal. Project leader Charlotte then set the scene for the meeting, with a list of suggested key outcomes for the two days in order to guide discussions. These are shown below:

- 1. Selection of key traits/methods for characterization in project
- 2. Collation of current practice of wild Daucus conservation and viability monitoring
- 3. Collation of data on global wild Daucus collections
- 4. Consider requirements for a global conservation strategy for Daucus

Current management practices of Daucus CWR in genebanks

Ulrike gave a presentation on IPK Gatersleben and the wild *Daucus* material conserved there. She covered regeneration protocols and germination testing (using the Jacobsen Apparatus), including the variable nature of the germination test results of accessions following regeneration.

Charlotte noted that in her experience, accurate germination tests in this group of species are sometimes difficult under standard laboratory conditions. She noted that for wild accessions, the UK Vegetable Genebank now routinely tests germination over a 28-day period in compost in the glasshouse rather than an incubator as there is a perception that this gives a more reliable indication than incubator-based tests. She had contacted the wider Umbellifer Crops Working Group to ask for information on protocols employed by those not participating in the meeting, but had not had any responses. The issue of dormancy was also discussed, with the timing of germination tests after seed production potentially influencing the result. Treatments such as pre-soaking or pre-washing seed (the latter to remove compounds from the seed coat which inhibit germination) were also discussed.

Emmanuel and Charlotte decided to undertake tests in order to look more stringently at two factors affecting dormancy. Germination tests would be carried out as normal on regenerated material, but also on material which has been in long-term storage for 6 months as the elapsed time may have allowed seed to move to a different stage in the dormancy cycle. The impact of soaking and washing seed prior to testing should be investigated as well as direct comparisons of glasshouse vs standard ISTA incubator based tests. Charlotte and Emmanuel agreed to take this forward during the course of the project. The results will be used to inform a suggested 'best practice' document.

Summary of global ex situ collections of Daucus CWR

Charlotte provided a summary of global collections of wild *Daucus* germplasm for which data was accessible via Eurisco or Genesys. Other collections undoubtedly exist, but information is not available. She also provided a summary of the current collection at the Millennium Seed Bank (Kew) in the context of the ongoing Crop Wild Relative project. Ana and Violeta clarified the role of BPGV in the collecting phase of the Crop Wild Relative project. A summary of the Portuguese wild *Daucus* collection managed by BPGV was provided by Violeta. Emmanuel introduced the French *Daucus* network and the germplasm conserved within it. Charlotte also provided information reported by Chris Kik (CGN) relating to the collecting missions undertaken for *Daucus* CWR recently.

Consideration of a global conservation strategy for Daucus

This was briefly discussed in the context of ongoing activities and known collections. The link between in situ and ex situ conservation is not strong. Originally it was planned to invite representatives from the ECPGR Wild Species Conservation WG as well as Kew to discuss this topic,

however this was not possible due to problems with scheduling the meeting. The topic will be considered by the project partners over the next 12 months and representatives will be invited to the next meeting as appropriate.

Visit to BPGV

A very enjoyable and informative visit to BPGV was hosted by Ana and Violeta, with an introduction to the history of the genebank (40th anniversary!), a tour of the facilities and then a visit to the CarrotDiverse field trial plots.

Day 2 – 5th July 2017

Project protocols

Most of the day was spent discussing the exact protocols and descriptors to be used during the CarrotDiverse project.

Field Trials

Lists of descriptors in use had already been circulated by Ulrike, Annette and Violeta. The discussion focused on identifying priority descriptors for the latitude trial, general characterization and whether a minimum descriptor list for wild species could be identified for promotion within the wider Working Group. It was felt that a draft list could be circulated for comment, and also the results of the more detailed characterization within the project would inform whether the minimum descriptors list would be valid and useful.

For the more detailed phenotypic characterization required by the latitude trial, in-depth discussions were held on what was appropriate and feasible. Methods such as scanning leaves and seeds were discussed as potentially time-saving approaches. Emmanuel agreed to collate the agreed descriptors into one document, providing as much information on each one as possible such as protocols and photographs and to circulate it after the meeting.

Disease resistance screening

Thomas gave a presentation on JKI and his research and the image analysis protocols used. It was decided that the DNA for the GBS analysis would be extracted at JKI from the plants used for resistance testing, as it would enable the genotypes of the individuals tested to be assayed. The phenotypic data could be linked in at a population rather than individual level. He requires seed to be sent to him by October to allow for initial assessment to take place.

Violeta noted that some of the accessions conserved at BPGV had insufficient seed for further distribution but that seed from the same collecting mission and population may be conserved in France, or by the USDA as collection partners. Investigations into which accessions are duplicated and available should be undertaken.

Genotyping by Sequencing Analysis

Thomas described work looking at terpenes and marker association using GBS. Charlotte provided some information and preliminary data on results recently obtained at Warwick on diversity within vs between accessions in carrot.

Date of next meeting

It was felt that the next meeting should be held later in 2018 to allow for discussion of the acquired data.

The meeting closed and those present thanked Violeta and the BPGV for the excellent organization and hosting of the meeting.

List	of	Agreed	Actions
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Task	Partner	Anticipated Completion Date
Germination test Daucus CWR accessions	Emmanuel,	October 2018
to compare results after harvest and after	Charlotte	
~6 months in storage		
Test washing, soaking seed protocols	Emmanuel,	October 2018
with aim of removal of germination	Charlotte	
inhibitors from seed coats		
Carry out comparison of glasshouse and	Charlotte	October 2018
incubator protocols to identify which		
yields the most accurate results for		
monitoring seed health		
Align lists of accessions collected in	Emmanuel, Ana,	September 2017
Portugal which are duplicated in France,	Violeta	
additionally check status of material		
collected jointly with Phil Simon to		
ascertain seed stock levels and available		
characterization data		
Produce finalized list of accessions sown	Charlotte	Completed
in 2017		
Selection of 96 accessions for disease	All, Charlotte to	September 2017
resistance testing	coordinate	
Send seed to Thomas at JKI	All	October 2017
Produce list of descriptors for	Emmanuel	Completed
characterization and latitude trial with		
instructions and photographs		
Selection of accessions for general	All	December 2017
characterization in 2018 by December		
2017		