ECPGR Activity Grant Scheme – Phase IX – Fourth Call

Activity Report

Improving the characterization and conservation of umbellifer crop wild relatives in Europe

(CarrotDiverse)

July 2017 – September 2019

Compiled by the ECPGR Secretariat based on the final technical report of the University of Warwick and the final report of the European Genetic Resources (GenR 2017-3) project

August 2026

Contents

FINAL TECHNICAL REPORT – UNIVERSITY OF WARWICK

INTRODUCTION – PROJECT OUTLINE AND AIMS

Carrot (*Daucus carota sativus* L.) is the most economically significant of the eight crops which are the focus of the Umbellifer Crops Working Group (WG). Understanding the structure of crop wild relative (CWR) genepool diversity is an essential first set toward its effective conservation and use. A previous project in the WG focused on the identification of material for inclusion into AEGIS. The CarrotDiverse project built on some earlier work looking at carrot CWRs and use a collaborative phenotypic characterization programme in combination with Genotyping by Sequencing to identify agronomically significant traits and allelic variants. Ongoing work outside of ECPGR was also linked to in order to undertake preliminary work on the outline of a European component of a global integrated conservation strategy for carrot CWR. The original project aims were as follows:

1. Development of European contribution toward global conservation strategy for *Daucus* CWR (all project partners).
2. Characterization and Evaluation of *Daucus* CWR
3. Field-based phenotypic characterization
4. An evaluation of resistance of the selected material to common diseases of cultivated carrot
5. Genotyping of *Daucus* CWR accessions.

Specific project activities undertaken by the University of Warwick:

a. Overall co-ordination of the project, including first meeting (held in Braga, Portugal in June 2017) and second meeting (held in Zaragoza, Spain in November 2018)
b. Provision of seed from the UK Vegetable Genebank collections for characterization by project partners
c. Discuss and explore preliminary ideas concerning a global conservation plan for *Daucus* CWR, including optimisation of ex situ collection management and links with in situ conservation.
d. Genotyping by sequencing of 96 samples to understand genetic diversity within the wild D. *carota* genepool
e. Communication of project activities to the scientific community.

OUTCOMES AND OUTPUTS

Project coordination

Two meetings of the project were successfully co-ordinated, allowing common goals and approaches to be agreed, particularly on the characterization and evaluation aims. Meeting notes have been written up and are available on the project web page.

Contribution to the Global Conservation Strategy for *Daucus* CWR

It had been planned to work with organizations such as Royal Botanic Gardens, Kew in order to develop a better understanding of what is required for a global conservation plan for *Daucus* CWR. Due to personnel changes at Kew in both the first and second year of the project, it was not possible to invite representative to either meeting. Work was undertaken to draw existing data and studies together to understand the evidence available, as a starting point towards consideration of the requirements of such a strategy. Work to this end is still ongoing as a series of bilateral discussions and will continue as an in kind contribution to the Umbellifer Crops WG over the next months.

The concept of a potential global conservation plan was discussed among the project partners at the first meeting and preliminary data were gathered on global holdings of *Daucus* CWR in *ex situ* collections. Data from Genesys and EURISCO indicate that European genebanks conserve a significant proportion of total wild Daucus accessions (Figure 1). This is in agreement with Allender (2019) which provides a summary of global *Daucus* genetic resources in *ex situ* collections. The Daucus genus is in taxonomic flux and the nomenclature used to describe genebank accessions varies accordingly – see Appendix 1. Given the absence of representatives from Kew, discussions at the first meeting focused on what should be done in Europe toward ensuring high quality conservation of Daucus germplasm. Germination test protocols vary, yet are a key part of *ex situ* collection management. *Daucus* wild species often exhibit relatively low and variable germination compared to the cultivated form, yet the WG has not identified specific treatments to deal with issues such as seed dormancy and contamination but fungal spores. It was resolved that investigation into better protocols should be undertaken to deal with these issues and during the project, work was undertaken at the University of Warwick to understand the impact of seed coat sterilization and pre-soaking and whether these treatments led to improved and more accurate test results.

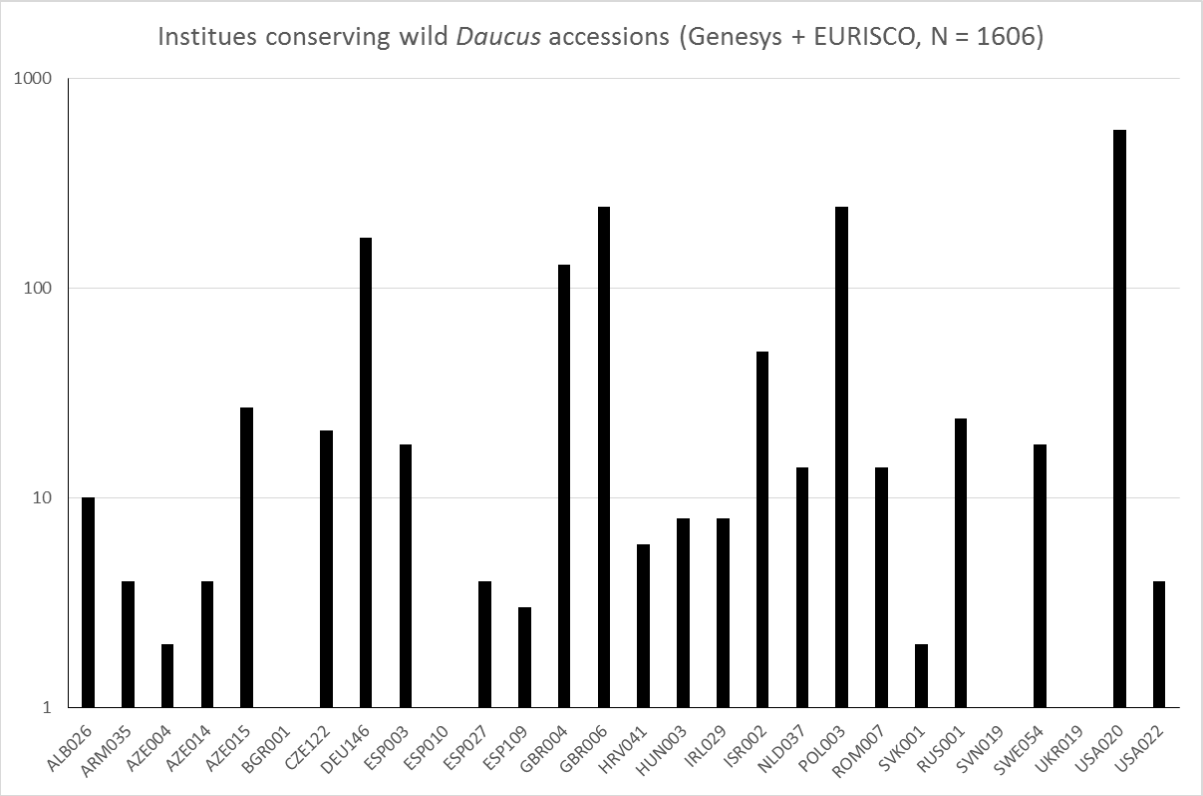

Figure 1. Wild *Daucus* germplasm in *ex situ* collections as reported by the Genesys database (https://www.genesys-pgr.org/ accessed April 2017). Note the log scale. Major collections outside of Europe are held in the USA.

A total of 31 accessions of *D. carota carota* were selected for germination testing. The germination test followed the standard procedure at the UK Vegetable Genebank; tests were carried out in two batches of 50 seeds per accession in an incubator set at 20°C. Seed were soaked in distilled water, or a sterilizing solution of Milton Fluid (2.5% sodium hypochlorite) with 1% Tween20 for one hour prior to setting up the germination tests. Untreated seed were used as a control. Seedlings were counted at 7 and 14 days, and a final additional count was undertaken at 28 days to determine if a two week protocol adequately captures viability. The results indicated that sterilization was beneficial, resulting in a higher % of seed counted as having successfully germinated (Figure 2). Extending the test protocol beyond the standard 14 days was beneficial for some accessions (Figure 3). These results will be presented to the WG as part of efforts to refine germination testing in the future.

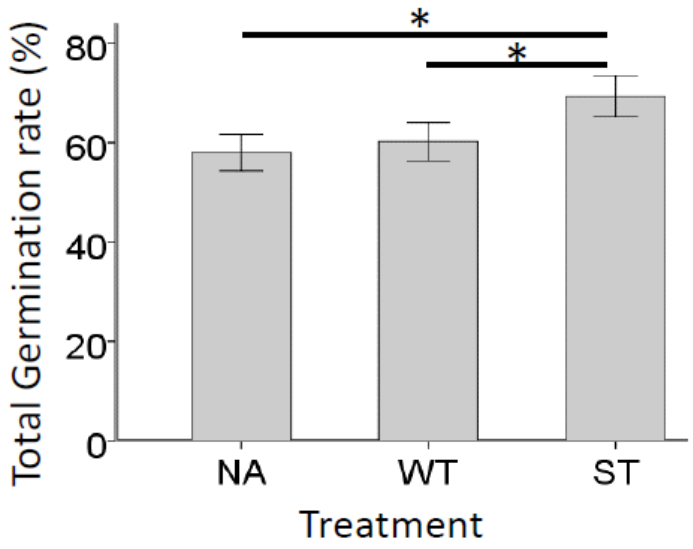

Figure 2. Sterilized seeds (ST) have a higher germination rate than water soaked (WT) and non treated (NA) seeds. * indicates significance ($p<0.05$), 95% confidence interval bars are shown.

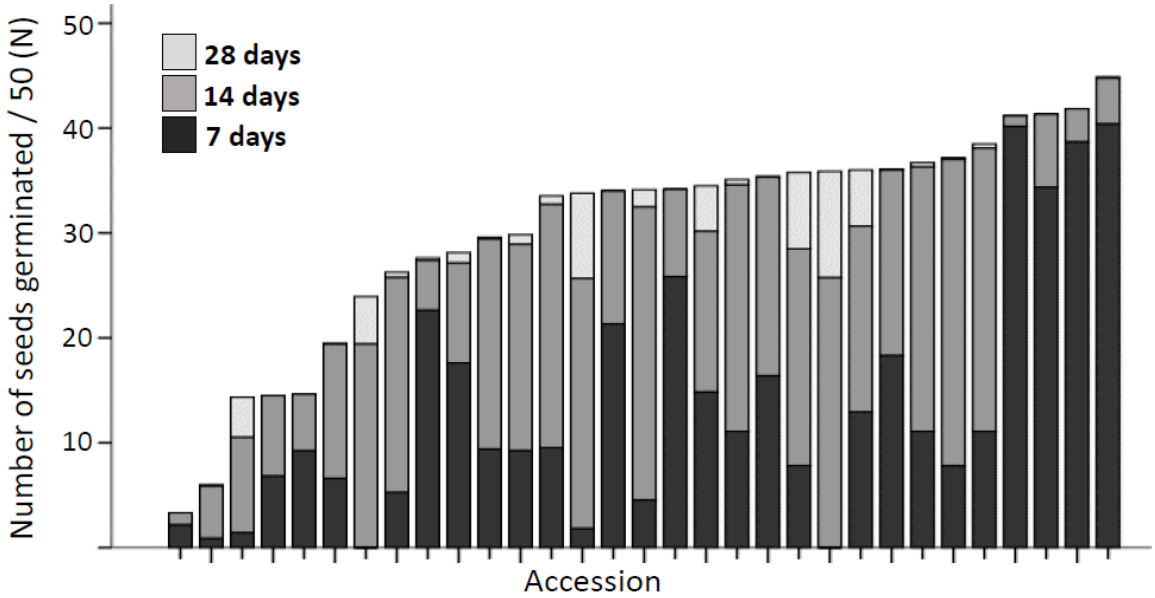

Figure 4. Overview of germination progression. Columns represent each accession. Germination is counted after 7 days (black), 14 days (grey) and 28 days (light grey). 50 seeds were placed on each dish.

Provision of seed for characterization activities

A total of 65 accessions were distributed to project partners in France, Sweden, Portugal and a further 26 to Germany for morphological characterization in field trials, evaluation for disease resitance and genotyping by sequencing. During the project, an additional evaluation exercise was undertaken; profiles of polyaceylenes and volatile organic compunds (VOCs) were obtained by researchers at the Julius Kuhn Institute. All material was transferred under the sMTA.

Genotyping by sequencing of 96 samples

This activity was undertaken by LGC, a commercial service provider, on DNA extracted from plants grown at the Julius Kühn Institute (JKI). This was undertaken in the Spring of 2019. Additional funding made available during the project enabled a further individual of each accession/line to be genotyped. The accessions for which genetype data were collected can be sen in Appendix 2, and also include some standard lines to enable comparison with pre-existing datasets.
Libraries from extracted DNA were prepapered by LGC and paired end sequencing of 150 base pairs using an Illumina platform was undertaken – this generated approximately 1.5M reads per sample. Bioinformatic analysis was undertaken by researchers at the JKI. A researcher from the University of Warwick travelled to JKI in August 2019 to receive training in the analysis of GBS data related association analyses (the costs associated with this are an in kind contribution to the project from both JKI and the University of Warwick), however a period of maternity leave has delayed this work.
Preliminary results are shown in Figure 5. The GBS data indicate five clear groups based around a combination of taxonomy and geography. There is an interesting separation between the *D. carota carota* samples from the Atlantic coasts of Europe and those from southern Europe, paricularly Portugal and Spain.

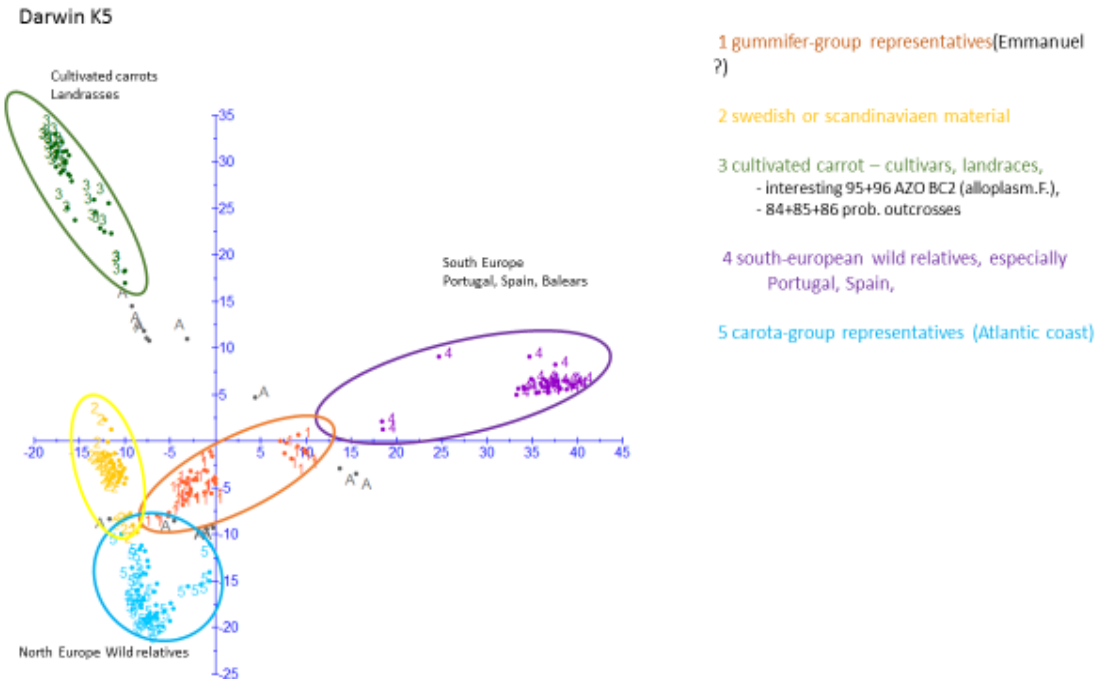

Figure 5. PCA plot of GBS data showing clustering into groups of common geographic or taxonomic origin. Each point represents an individual plant, more genetically similar individuals are located closer together. The analysis was undertaken by the Bioinformatics team at the JKI.

Communication of project activities to the scientific community

Charlotte Allender attended the ISHS Carrot and other Apiaceae Symposium in Krakow in September 2018, and presented the first year of the replicated phenotyping work undertaken in France, Portugal and Sweden. This has been written up as a paper for the conference proceedings and published in the journal Acta Horticulturae (Geoffriau et al, 2019). Other papers on the morphological characterization, GBS and evaluation activities will also be prepared in due course.

REFERENCES

Allender C (2019) Genetic resources for carrot improvement. In: *The Carrot Genome* (eds P. Simon, D. Grzebelus), Springer Nature, Switzerland

Geoffriau, E., Charpentier, T., Huet, S., Hägnefelt, A., Lopes, V., Nothnagel, T., Lohwasser, U., Mallor Gimenez, C. and Allender, C. (2019). CarrotDiverse: understanding variation in a wild relative of carrot. Acta Hortic. 1264, 151-156. DOI: 10.17660/ActaHortic.2019.1264.18

APPENDIX 1

Taxonomic designations of wild *Daucus* material stored in *ex situ* collections as reported by Genesys (https://www.genesys-pgr.org/ accessed April 2017)

Taxon	No. of accessions	Taxon	No. of accessions
Daucus carota	646	*Daucus carota* var. *sulfureus*	1
Daucus carota carota x	1	*Daucus aureus*	22
Daucus carota gingidium?	1	*Daucus bicolor*	23
Daucus carota gummifer x	1	*Daucus broteri*	44
Daucus carota subsp. *azoricus*	6	*Daucus capillifolius*	12
Daucus carota subsp. *carota*	310	*Daucus crinitus*	25
Daucus carota subsp. *commutatus*	10	*Daucus durieua*	2
Daucus carota subsp. *drepanensis*	2	*Daucus glaber*	7
Daucus carota subsp. *fontanesii*	2	*Daucus glochidiatus*	3
Daucus carota subsp. *gadecaei*	2	*Daucus gracilis*	3
Daucus carota subsp. *gadecei*	1	*Daucus guttatus*	33
Daucus carota subsp. *gummifer*	27	*Daucus halophilus*	1
Daucus carota subsp. *halophilus*	8	*Daucus hispidifolius*	1
Daucus carota subsp. *hispanicus*	6	*Daucus* hybr.	7
Daucus carota subsp. *hispidus*	4	*Daucus involucratus*	9
Daucus carota subsp. *major*	10	*Daucus littoralis*	4
Daucus carota subsp. *majoricus*	1	*Daucus mauritii*	1
Daucus carota subsp. *maritimus*	27	*Daucus montanus*	1
Daucus carota subsp. *maxima*	3	*Daucus montividensis*	1
Daucus carota subsp. *maximus*	40	*Daucus muricatus*	51
Daucus carota subsp. *rupestris*	1	*Daucus pusillus*	45
Daucus carota subsp. *sativus*	16	*Daucus sahariensis*	7
Daucus carota var. *albus*	22	*Daucus* sp.	134
Daucus carota var. *aurantius*	1	*Daucus syrticus*	12
Daucus carota var. *minor*	1	*Daucus tenuisectus*	2

APPENDIX 2

Accessions and standard lines selected for GBS analysis

Species / subspecies	Accession No.	Origin
Daucus carota	BPGV 08411	BPGV
Daucus carota	BPGV 08391	BPGV
Daucus carota	BPGV 08159	BPGV
Daucus carota	BPGV 08412	BPGV
Daucus carota	BPGV 08427	BPGV
Daucus carota	BPGV 08610	BPGV
Daucus carota	BPGV 08429	BPGV
Daucus carota	BPGV 16273	BPGV
Daucus carota	BPGV 08432	BPGV
Daucus carota	BPGV 16295	BPGV
D.c. carota ssp. *maritimus*	625	PRT
D.c. carota ssp. *carota*	645	PRT
D.c. carota ssp. *maximus*	652	PRT
D.c. gummifer ssp. *hispanicus*	683	FRANCE
D.c. gummifer ssp. *hispanicus var.hispanicus*	684	FRANCE
D.c. gummifer ssp. *hispanicus*	686	FRANCE
D.c. gummifer ssp. *hispanicus*	695	FRANCE 2017
D.c. gummifer ssp. *hispanicus*	695	FRANCE 2018
D.c. carota ssp. *carota var. carota*	699	FRANCE
D.c. carota ssp. *carota var. carota*	704	FRANCE
D.c. carota ssp. *maritimus*	706	FRANCE
D.c. gummifer ssp. *hispanicus var.hispanicus*	709	FRANCE
D.carota var. gummifer	710	FRANCE
D.c. gummifer ssp. *gummifer var. gummifer*	711	FRANCE
D. carota ssp. *gadecaei*	715	FRANCE
D.c. gummifer ssp. *gummifer var. gummifer*	720	FRANCE
D.c. gummifer ssp. *gummifer var. gummifer*	732	FRANCE
D.c. gummifer ssp. *gummifer var. gummifer*	735	FRANCE
D.c. gummifer ssp. *gummifer var. gummifer*	740	FRANCE
D.c. carota ssp. *carota var. carota*	743	FRANCE
D.c. ssp. *gadecaei*	761	FRANCE
D.c. gummifer ssp. *drepanensis*	780	FRANCE
D.c. gummifer ssp. *commutatus*	786	FRANCE
D.c. gummifer ssp. *gummifer var. fontanesii*	790	FRANCE
D.c. carota ssp. *carota var. mauritanicus*	791	FRANCE
D.c .carota ssp. *carota var. carota*	800	FRANCE
D.c .carota ssp. *carota var. carota*	801	FRANCE
D.c. carota ssp. *maritimus*	802	FRANCE
D.c. carota ssp.*?*	806	FRANCE
D.c. gummifer ssp. *gummifer var. fontanesii*	807	FRANCE

Species / subspecies	Accession No.	Origin
D.c .carota ssp. *carota var. carota*	821	ITA
D.c .carota ssp. *carota var. carota*	822	ITA
D.c .carota ssp. *carota var. carota*	880	FRANCE
D.carota var. carota	886	FRANCE
Daucus carota L. sg *gummifer*	891	FRANCE
Daucus carota L.	NGB 16882	Sweden
Daucus carota L.	NGB 21129	Denmark
Daucus carota L.	NGB 21386	Norway
Daucus carota L.	NGB 21387	Sweden
Daucus carota L.	NGB 21389	Sweden
Daucus carota L.	NGB 21720	Denmark
Daucus carota L.	NGB 21878	Denmark
Daucus carota L.	NGB 21924	Denmark
Daucus carota L.	NGB 21956	Sweden
Daucus carota L.	NGB 23757	Denmark
Daucus carota ssp. *sativus* ((Hoffm.) Arcangeli.)	NGB 23926	Denmark
Daucus carota ssp. *sativus* ((Hoffm.) Arcangeli.)	NGB 24337	Sweden
Daucus carota L.	NGB 24352	Norway
Daucus carota L.	NGB 24386	Denmark
Daucus carota L.	NGB 25816	Denmark
Daucus carota carota	HRI 13:006666	Wellesbourne
Daucus carota/drepanensis	HRI 13:006668	Wellesbourne
Daucus carota	HRI 13:006673	Wellesbourne
Daucus carota carota	HRI 13:006678	Wellesbourne
Daucus carota carota	HRI 13:006679	Wellesbourne
Daucus carota carota	HRI 13:006680	Wellesbourne
Daucus carota carota	HRI 13:007161	Wellesbourne
Daucus carota carota	HRI 13:007191	Wellesbourne
Daucus carota carota	HRI 13:007877	Wellesbourne
Daucua *carota commutatus*	HRI 13:007999	Wellesbourne
Daucus carota carota	HRI 13:008001	Wellesbourne
Daucus carota carota	HRI 13:008002	Wellesbourne
Daucus carota carota	HRI 13:008692	Wellesbourne
Daucus carota carota	HRI 13:008696	Wellesbourne
Daucus carota carota	HRI 13:008698	Wellesbourne
Daucus carota carota	HRI 13:008704	Wellesbourne
Daucus carota gummifer	HRI 13:008706	Wellesbourne
Daucus carota carota	HRI 13:008708	Wellesbourne
Daucus carota carota	HRI 13:008716	Wellesbourne

Species / subspecies	Accession No.	Origin
Daucus carota gingidum	HRI 13:008728	Wellesbourne
Daucus carota maritimus	HRI 13:009217	Wellesbourne
Daucus carota carota	HRI 13:009230	Wellesbourne
Daucus carota carota	HRI 13:010407	Wellesbourne
Daucus carota carota	HRI 13:011165	Wellesbourne
Daucus carota carota	HRI 13:013208	Wellesbourne
Daucus carota carota	HRI 13:013212	Wellesbourne
Daucus carota sativus	Rotin	Sperli
Daucus carota sativus	Nerac	Bejo
Daucus carota sativus	Presto	Vilmorin
Daucus carota sativus	Brasilia (DU 8/12-2)	USDA
Daucus carota sativus	Senta	JKI - collection
Daucus carota sativus	Gosun	JKI - collection
Daucus carota sativus	Nevis	Bejo
Daucus carota sativus	Bolero	Bejo
Daucus carota sativus	Mh12/16-12	JKI - collection
Daucus carota sativus	507/98-95 x 511/98-10	JKI - collection

EXTRACTS FROM THE FINAL REPORT OF THE GENRES 2017-3 PROJECT[1]

Introduction

With Project GenR 2017-3, the German Federal Ministry for Food and Agriculture supported four ECPGR activities that were positively evaluated by the ECPGR Executive Committee under the Third and Fourth Call for proposals of the ECPGR Activity Grant Scheme in ECPGR Phase IX. Among these, the CarrotDiverse activity aimed at the provision of selected carrot wild relatives phenotypic and genotypic data to EURISCO, within the framework of the definition of a coherent strategy to adequately conserve the Daucus complex gene pool in Europe.

Project expert meetings

Expert meeting in Braga, Portugal

The first expert meeting was held 4-5 July 2017 at the Banco Português de Germoplasma Vegetal (BGPV) in Braga, Portugal. The meeting covered two broad areas: discussion and agreement on project protocols and the European and global landscape of the project in the context of other significant collections and activities on wild *Daucus* species. Five project partners from France, Germany, Portugal, the UK and five local observers attended the meeting. The workshop web page with presentations, pictures and the full workshop report is available from the ECPGR website (here).

Expert meeting in Zaragoza, Spain

The second meeting was held from 13-14 November 2018 and was hosted by Cristina Mallor-Gimenez at CITA. It was attended by Charlotte Allender, Emmanuel Geoffriau, Annette Hägenfelt and Thomas Nothnagel. Violeta Lopes joined the discussion on characterization via videoconference link from Braga. The discussions covered aspects of the characterization trial, including common problems with assessments. It was noted that the two seasons had produced very different results.
The meeting offered an opportunity to raise awareness of the CITA vegetable genebank (BGHZ) and the cultivated carrot and other vegetable collections conserved there. The context of the genebank in relation to the Spanish national conservation activities was also highlighted.

Project achievements

Field-based characterization of ca. 60 accessions across three sites (morphology, taxonomy, reproductive biology, environmental adaptation); including evaluation of material selected for resistance to diseases of cultivated carrot.
Characterization and taxonomical verification of 30 accessions (15 from France and 15 from the UK) were carried out in a replicated trial in Angers (France), Braga (Portugal) and Alnarp (Sweden) in 2017. Field characterization was carried out again at three trial locations in 2018 (France, Portugal and Sweden). These 2018 trials had two components:

1. A repeated replicated field trial of the ten accessions grown across all three sites to identify descriptors which are robust to environmental influence. Traits, including leaf characters, flowering behaviour and seed morphology, were studied.
2. A total of 80 accessions were grown for basic phenotypic characterization (30 accessions at Braga and Angers, 20 at NordGen). These accessions were grown at a single site only, with no replication.

In all cases, germplasm was sourced from the French *Daucus* Network, the UK Vegetable Genebank and NordGen collections.

Green-house phenotyping and evaluation of 96 accessions for resistance to diseases of cultivated carrot.

[1] The additional information on CarrotDiverse provided here is taken from the final report (March 2020) prepared by the ECPGR Secretariat for the European Genetic Resources (GenRes 2017-3) project, funded by the German Federal Ministry of Food and Agriculture.

Accessions of wild carrots to be evaluated for resistance to diseases of cultivated carrot from project partners were transferred to JKI, Quedlinburg. Ten accessions were sent from Portugal, 35 from France, 15 from the Nordic countries and 26 from the UK. Overall, 96 accessions (including ten cultivated carrot standards from Germany) were subjected to inoculation (up to six individuals of each accession or line) with three species of fungal pathogen (*Alternaria alternata, A. daucii* and *A. radicina*). Resistance was scored using image analysis of leaf sections or petioles as appropriate. All accessions (in total 886 plants) were grown in greenhouse conditions and evaluated for 23 phenotypic traits. Two plants of each accession (=192 plants) were randomly selected for Genotyping by Sequencing (GBS) analysis (see below). The same GBS set was also analyzed for polyacetylene content (see below). After a pre-evaluation step to verify the growing reaction of the plants, disease resistance tests were carried out in July 2018.

Analysis of the level of polyacetylene in the roots of 200 samples
As indicated above, a total of 96 accessions or lines were sown at JKI in July 2018 for the first stages of disease resistance evaluation. Root material from two plants per accession (total 191 plants) was processed with standard procedures, and HPLC analyses of polyacetylene compounds were carried out, with specific measurement of profiles for Falcarinidiol, 3-O-Acetyl-falcarinidiol and Falcarinol.

Genotyping-by-sequencing analysis of 96 samples selected from plants scored during phenotypic characterization
At JKI, genomic DNA was extracted from leaf material (two individuals each) of the 96 accessions/lines and sent to LGC genomics, Berlin, Germany, for library construction and normalized GBS. The SNP raw data were then prepared and analyzed at JKI. These samples can be matched to individual disease resistance scores and polyacetylene profiles, allowing a future understanding of the relationship between genotype and phenotype.

Accomplishments towards expected outcomes

Outline of European contribution towards a global strategy for the conservation of *Daucus* CWR
It had been planned to work with organizations such as Royal Botanic Gardens, Kew in order to develop a better understanding of what is required for a global conservation plan for *Daucus* CWR. Due to personnel changes at Kew in both the first and second year of the project, it was not possible to invite a representative to either meeting. Work was undertaken to draw existing data and studies together to understand the evidence available, as a starting point towards consideration of the requirements of such a strategy. Work to this end is still ongoing as a series of bilateral discussions and will continue as an in-kind contribution to the Umbellifer Crops WG over the next months.
The concept of a potential global conservation plan was discussed among the project partners at the first meeting, and preliminary data were gathered on global holdings of *Daucus* CWR in *ex situ* collections. Data from Genesys and EURISCO indicate that European genebanks conserve a significant proportion of total wild *Daucus* accessions. The *Daucus* genus is in taxonomic flux, and the nomenclature used to describe genebank accessions varies accordingly. Given the absence of representatives from Kew, discussions at the first meeting focused on what should be done in Europe toward ensuring high-quality conservation of *Daucus* germplasm. Germination test protocols vary, yet are a key part of *ex situ* collection management. *Daucus* wild species often exhibit relatively low and variable germination compared to the cultivated form, yet the WG has not identified specific treatments to deal with issues such as seed dormancy and contamination by fungal spores. It was resolved that an investigation into better protocols should be undertaken to deal with these issues, and during the project, work was undertaken at the University of Warwick to understand the impact of seed coat sterilization and pre-soaking and whether these treatments led to improved and more accurate test results.
Work has been undertaken within CarrotDiverse to understand and improve the recommended protocol for germination testing of wild carrot material – correctly understanding seed stock viability is a key aspect of germplasm management, and up to 15 accessions which have been stored for differing lengths of time, have been tested with pre-treatments to improve germination tests (surface sterilization and pre-soaking). The results indicated that sterilization was beneficial, resulting in a higher percentage of seed counted as having successfully germinated. Extending the test protocol beyond the standard 14

days was beneficial for some accessions. These results will be presented to the WG as part of efforts to refine germination testing in the future. This information is vital to understand how best to handle this germplasm *ex situ* in order to contribute toward adequate global conservation.
The project has also delivered a key list of minimum descriptors for consideration when characterization is being undertaken in the future.

Identification of a panel of key Daucus CWR accessions from European genbanks for inclusion in the European Collection
The identification of a panel of key *Daucus* CWR accessions from European genebanks for inclusion in the European Collection will be possible after completion of the analysis of characterization data and the consideration of seed stock and viability.

Characterization data (morphology, disease resistance) from three locations for 60 accessions of Daucus CWR and associated genotypic data on these accessions, made available in EURISCO
Data collection was completed; data are currently being collated and will be formatted to permit upload into EURISCO for the benefit of users and other collection managers. Upload will take place following agreed protocols involving the relevant National Coordinator.

Green-house phenotyping and evaluation of 96 accessions for resistance to diseases of cultivated carrot
During phenotyping, three 'wild' accessions expressing roots similar to the controls were identified as landraces, as opposed to wild relatives. Other two wild accessions expressed relatively large white storage roots, suggesting possible outcrosses. Morphological characterization of 86 accessions showed great variability compared to the cultivated controls. Also, 38% of the carrot wild relatives' samples reached the flowering phase (bolting) without vernalization and were evaluated for additional traits. Surprisingly, a relatively high percentage of the plants showed male sterility of various types. The bioassays for disease resistance detected disease symptoms with significant differences in the severity scores. A number of wild relatives showed lower disease severity scores compared to the controls.

Evaluation of 200 samples of wild carrot for polyacetylene content, a compound reported to have both positive and negative traits for human consumption, depending on its level
The 96 accessions (up to two individuals per accession, total of 172 wild individuals plus standard lines) were assessed for three major polyacetylenes (PA), which were identified and quantified in the root tissues. The three PAs amounts correlate with each other, but no correlation was detectable between the PAs and the data sets of the resistance screening. During the project, an additional evaluation exercise was undertaken; profiles of polyacetylenes and volatile organic compounds (VOCs) were obtained by researchers at the Julius Kuhn Institute.

Genotyping using the GBS protocol of 96 samples of wild carrot
GBS paired end sequencing of 150 base pairs using an Illumina platform was undertaken – this generated approximately 1.5M reads per sample. In a preliminary data analysis, genetic distances and population structure were determined on a reduced marker data set. Among others, a principal coordinate analysis was carried out and is reported below (Fig 1), showing the clear structuration of the crop wild relatives originated from different geographic zones and their separation from the cultivated controls.

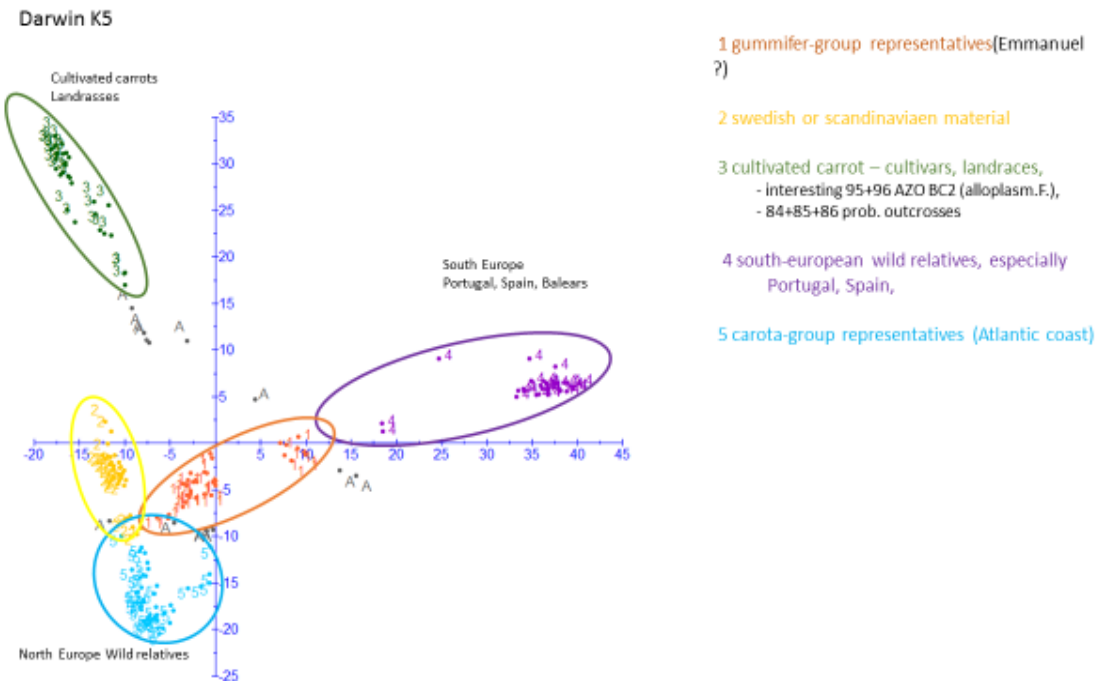

Figure 1. PCA plot of GBS data showing clustering into groups of common geographic or taxonomic origin. Each point represents an individual plant, more genetically similar individuals are located closer together. The analysis was undertaken by the Bioinformatics team at the JKI.

The combined GBS/characterization and polyacetylene/VOC dataset is very large and will take a significant effort to analyze it fully; however, this work will be undertaken after the end of the project and the results submitted for publication. The association study GBS-PA will focus on existing candidate genes. Also, candidate genes probably involved in carrot bolting and flowering processes will be tested for association with the bolting behaviour of the GBS set. The identification of potential resistance genes will also be the subject of study, and the CarrotDiverse data set will be used for the identification and verification of candidates.

Results valorization and publications

A communication[2] has been presented at an International symposium and the basis for an article on the latitude effect on wild carrot morphology and descriptors has been prepared. Other publications on genetic diversity and GWAS are under discussion. All data sets will be prepared for inclusion into EURISCO.

Appraisal by Bioversity/ECPGR Secretariat of results obtained

The Umbellifer crops WG focused on wild relatives. Data from field tests carried out in three locations still need to be thoroughly analyzed in order to provide recommendations on the most useful descriptors and methodologies. Phenotypic data will also be matched with genotypic, biochemical and bioassay disease resistance data obtained in the laboratory. Genetic data have shown clear clustering of the analyzed accessions into groups of common geographic or taxonomic origin. The bioassays for disease resistance detected promising accessions affected by lower disease severity compared to the controls. The concept of a potential global conservation plan was discussed among the project partners and preliminary data on global holdings of *Daucus* CWR in *ex situ* collections indicate that European

[2] Geoffriau, E., Charpentier, T., Huet, S., Hägnefelt, A., Lopes, V., Nothnagel, T., Lohwasser, U., Mallor Gimenez, C. and Allender, C. (2019). CarrotDiverse: understanding variation in a wild relative of carrot. Acta Hortic. 1264, 151-156. DOI: 10.17660/ActaHortic.2019.1264.18

genebanks conserve a significant proportion of total wild *Daucus* accessions. New tests on germination protocols gave good indications on the possibility to improve germination of wild material, which is a key factor for the efficient long-term conservation of carrot wild relatives in genebanks and their use.
The budget planned for this project was entirely utilized, also generating additional activities, originally unplanned, some of them also carried out as in-kind inputs (germination tests at Warwick University).