

# CarrotDiverse

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



Federal Office for  
Agriculture and Food

**Tuesday 4 July 2017**

09:00-09:30	<p><b>Welcome</b></p> <p>On behalf of the INIAV's President and of the National Coordinator of ECPGR</p> <p>Introduction to the meeting</p>	<p>Ana Maria Barata</p> <p>Charlotte Allender</p>
09:30-11:00	<p><b>Current management practices of <i>Daucus</i> CWR in genebanks</b></p> <ul style="list-style-type: none"> <li>• Viability monitoring</li> <li>• Management practices</li> <li>• Aim to produce suggested best practice guide for this group</li> </ul>	<p>Charlotte, Ulrike, Violeta, Emmanuel, discussion by all</p>
11:00–11:30	<p><i>Coffee break</i></p>	
11:30-12:15	<p><b>Summary of global <i>ex situ</i> collection of <i>Daucus</i> CWR</b></p> <ul style="list-style-type: none"> <li>• Report of data available via Genesys, Eurisco</li> <li>• Portuguese Collection</li> <li>• Other collections – France, Kew, CGN</li> </ul>	<p>Charlotte, Violeta Lopes Emmanuel,</p>
12:15-13:00	<p>Crop Trust/Kew – CWR project</p> <ul style="list-style-type: none"> <li>• Previous work, Current status, pre breeding</li> </ul> <p>Consideration of global conservation strategy for <i>Daucus</i></p> <ul style="list-style-type: none"> <li>• Discussion of approach and information required</li> </ul>	<p>Introduction by Charlotte, discussion by all</p> <p>Introduction by Charlotte, discussion by all</p>
13:00-14:00	<p><i>Lunch break</i></p>	
14:00-17:00	<p><b>Visit to BPGV and Carrot diverse trials</b></p> <p>BPGV ( activities and visit)</p> <p>Field visit to observation of phenotype and latitude trait studies</p>	<p>Ana Maria Barata</p> <p>Violeta Lopes and all</p>
17:00	<p><b>Summary of Day 1 – key points recorded</b></p>	<p>Charlotte Allender and all</p>
<i>Evening</i>	<p><b><i>Social dinner</i></b></p>	

**Wednesday 5<sup>th</sup> July 2017**

09:00-09:30	<p>CarrotDiverse project</p> <ul style="list-style-type: none"> <li>• Aims and objectives</li> <li>• Expected outputs of the meeting</li> </ul>	Charlotte
09:30-11:00	<p>Project protocols – discussion</p> <ul style="list-style-type: none"> <li>• Exact traits to score</li> <li>• Frequency of observations</li> <li>• Digital imaging standards</li> <li>• Disease resistance</li> </ul>	Emmanuel, Thomas, discussion by all
11:00-11:30	<i>Coffee break</i>	
11:30-13:00	<p>Project protocols – discussion (continued)</p> <ul style="list-style-type: none"> <li>• Genotyping by sequencing (introduction)</li> <li>• Selection of individuals</li> <li>• DNA extraction requirements and collation of samples</li> </ul>	Emmanuel, Charlotte, discussion by all
13:00-14:00	<i>Lunch</i>	
14:00-16:30	<p>Project protocol discussion (continued)</p> <ul style="list-style-type: none"> <li>• Finalization of protocol document</li> <li>• Update of project timeline</li> <li>• Consideration of next meeting</li> <li>• Agreement of meeting report</li> </ul>	Charlotte, discussion by all
16:30	<i>Closure of meeting and departures</i>	

# Meeting Aims

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*

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# Management of wild *Daucus* germplasm in long term storage

- Collection/regeneration discussed at previous WG meetings
- Viability testing protocols
  - What protocols are used (ISTA, others?)
  - Pretreatments/sterilisation?
  - De-awning seed?
  - Viability thresholds?

# Viability Monitoring

- Wild *Daucus* accessions can show low viability under ISTA test conditions
  - How do different collections monitor viability?
  - Can we identify best practice for these taxa?

# About the UK Vegetable Genebank

**A repository for the conservation of vegetable crop genetic diversity**

14,000 seed samples

47 vegetable and herb crops

520 million seeds

Our collections are globally significant



Seed originates from 108 countries





# Crops we work with



Total number of Accessions	14022
<i>Brassica</i> (cabbage and related crops)	5273
<i>Allium</i> (onions, leeks)	1754
<i>Daucus</i> (carrot)	1481
<i>Lactuca</i> (lettuce)	1483
<i>Raphanus</i> (radish)	803



# What we do

- **Conservation activity**
  - Seed storage at  $-20^{\circ}\text{C}$
  - Monitor germination
  - Regeneration of samples
- **Seed distribution**
- **Information management**
- **UK and international collaboration**



# Bespoke facilities for seed conservation

- 2 seed stores (-20°C) - ~90m<sup>2</sup>
- 2 drying rooms (15% RH and 15°C)
- Seed cleaning facilities
- 1 glasshouse (year round growth)
- 2 glasshouses (46 isolation cages)
- Pollinator production facilities
- Cold storage facilities for onions/roots



# UK Vegetable Genebank

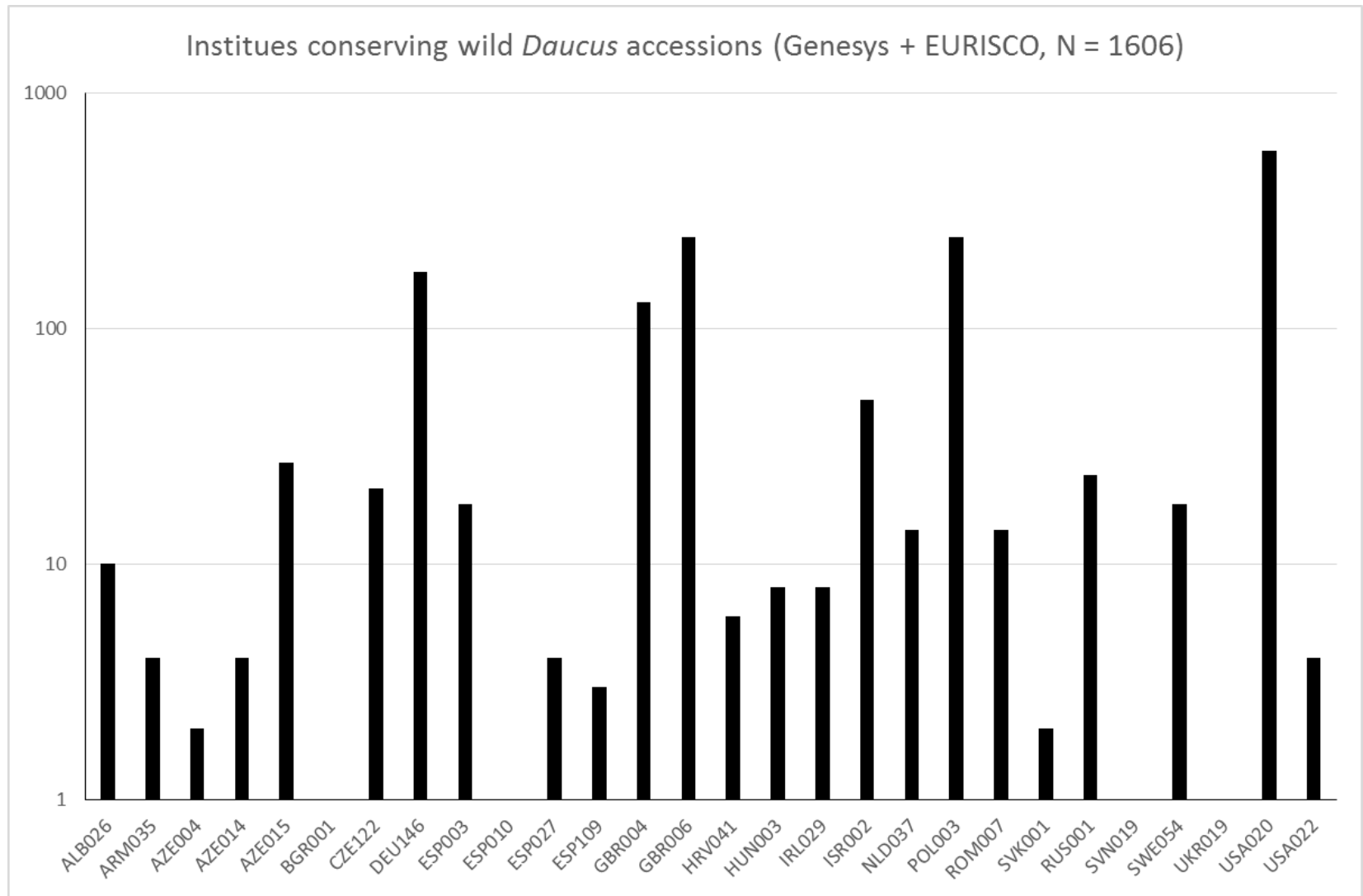
- 247 wild *Daucus* accessions
  - Ongoing taxonomic verification, previous ECPGR projects
- Chill pre-treatment, 100 seeds used in germination tests
  - Most wild *Daucus* tested in glasshouse/compost
  - Poor results in ISTA protocol (incubator based tests)
  - Germination scored after 28 days, can be left for longer if necessary
  - Any other suggestions would be helpful....

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# Global wild *Daucus* germplasm collections

- Genesys/EURISCO used to get total accessions
  - Missing other collections (Embrapa, China, others?)
  - Differences Genesys and EURISCO
  - Other European collections not listed (France, Portugal, CGN...)
  - Recent work by Crop Trust has taken an overview but may be missing finer details

# Genesys and Eurisco data – some differences in numbers of accessions at some institutes but broadly comparable datasets (+USDA)



## Breakdown of Genesys *Daucus* CWR records by taxon -

<i>Daucus carota</i>	646	<i>Daucus aureus</i>	22
<i>Daucus carota carota</i> x	1	<i>Daucus bicolor</i>	23
<i>Daucus carota gingidium?</i>	1	<i>Daucus broteri</i>	44
<i>Daucus carota gummifer</i> x	1	<i>Daucus capillifolius</i>	12
<i>Daucus carota</i> subsp. <i>azoricus</i>	6	<i>Daucus crinitus</i>	25
<i>Daucus carota</i> subsp. <i>carota</i>	310	<i>Daucus durieua</i>	2
<i>Daucus carota</i> subsp. <i>commutatus</i>	10	<i>Daucus glaber</i>	7
<i>Daucus carota</i> subsp. <i>drepanensis</i>	2	<i>Daucus glochidiatus</i>	3
<i>Daucus carota</i> subsp. <i>fontanesii</i>	2	<i>Daucus gracilis</i>	3
<i>Daucus carota</i> subsp. <i>gadecaei</i>	2	<i>Daucus guttatus</i>	33
<i>Daucus carota</i> subsp. <i>gadecei</i>	1	<i>Daucus halophilus</i>	1
<i>Daucus carota</i> subsp. <i>gummifer</i>	27	<i>Daucus hispidifolius</i>	1
<i>Daucus carota</i> subsp. <i>halophilus</i>	8	<i>Daucus hybr.</i>	7
<i>Daucus carota</i> subsp. <i>hispanicus</i>	6	<i>Daucus involucratus</i>	9
<i>Daucus carota</i> subsp. <i>hispidus</i>	4	<i>Daucus littoralis</i>	4
<i>Daucus carota</i> subsp. <i>major</i>	10	<i>Daucus mauritii</i>	1
<i>Daucus carota</i> subsp. <i>majoricus</i>	1	<i>Daucus montanus</i>	1
<i>Daucus carota</i> subsp. <i>maritimus</i>	27	<i>Daucus montividentis</i>	1
<i>Daucus carota</i> subsp. <i>maxima</i>	3	<i>Daucus muricatus</i>	51
<i>Daucus carota</i> subsp. <i>maximus</i>	40	<i>Daucus pusillus</i>	45
<i>Daucus carota</i> subsp. <i>rupestris</i>	1	<i>Daucus sahariensis</i>	7
<i>Daucus carota</i> subsp. <i>sativus</i>	16	<b>Daucus sp.</b>	<b>134</b>
<i>Daucus carota</i> var. <i>albus</i>	22	<i>Daucus syrticus</i>	12
<i>Daucus carota</i> var. <i>aurantius</i>	1	<i>Daucus tenuisectus</i>	2
<i>Daucus carota</i> var. <i>minor</i>	1		
<i>Daucus carota</i> var. <i>sulfureus</i>	1		

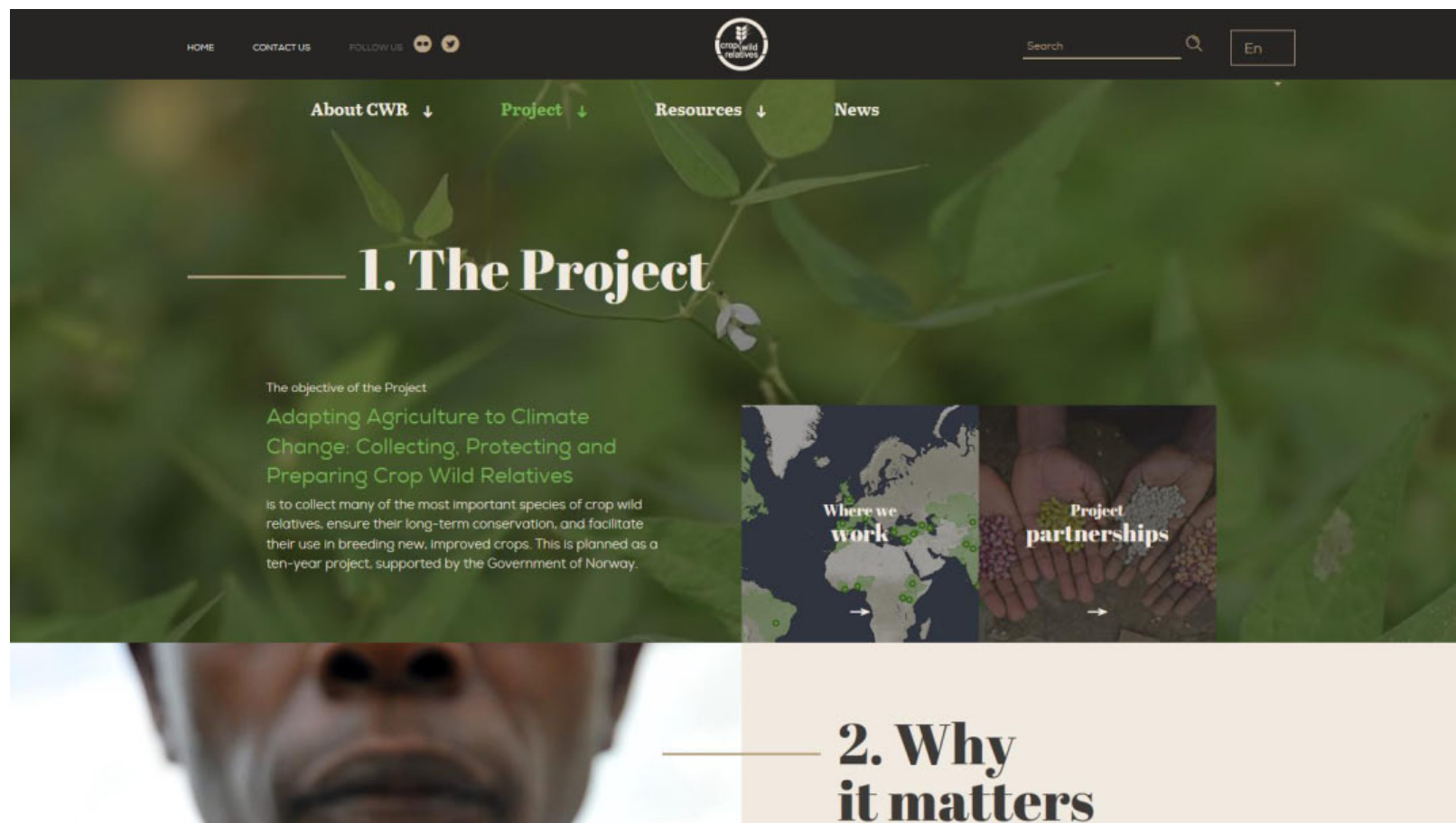


# Other collections - CGN

- Collecting expeditions funded jointly by Dutch government and consortium of breeding companies
- *Daucus* collected from Uzbekistan and Kyrgyzstan in 2015
  - 22 landraces
  - 90 wild populations
- Available under sMTA from 5 years after first regeneration (2021 onwards)

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# Global Activity on *Daucus* CWR



The screenshot shows the website's navigation bar with links for HOME, CONTACT US, FOLLOW US, and a search bar. The main content area features a green background with a plant stem. The section is titled "1. The Project" and includes the following text:

The objective of the Project  
**Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives**  
is to collect many of the most important species of crop wild relatives, ensure their long-term conservation, and facilitate their use in breeding new, improved crops. This is planned as a ten-year project, supported by the Government of Norway.

Two images are shown: "Where we work" (a world map) and "Project partnerships" (hands holding seeds).

Below the main content, a section titled "2. Why it matters" is partially visible, with a blurred image of a person's face on the left.

<http://www.cwrdiversity.org>

# Activity on Daucus CWR in project – location of partner institutes for collecting and pre-breeding activities



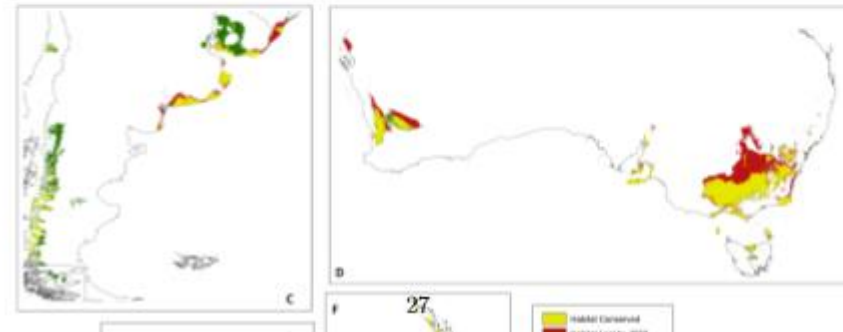
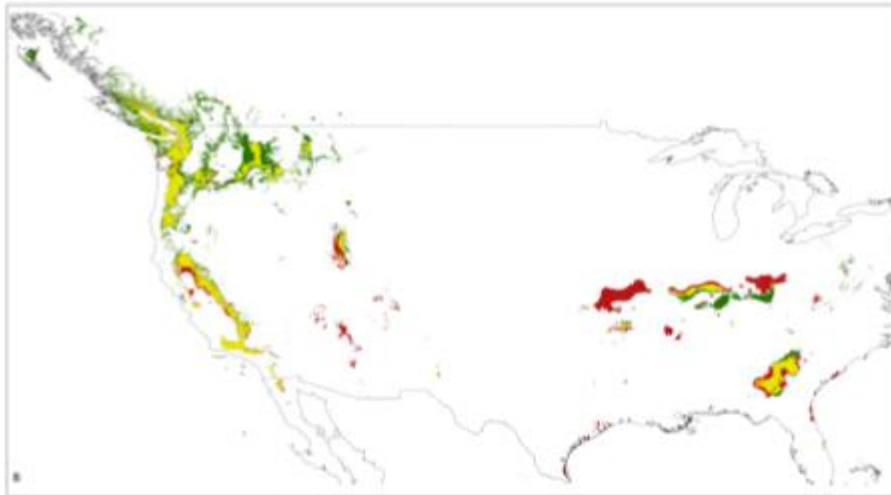
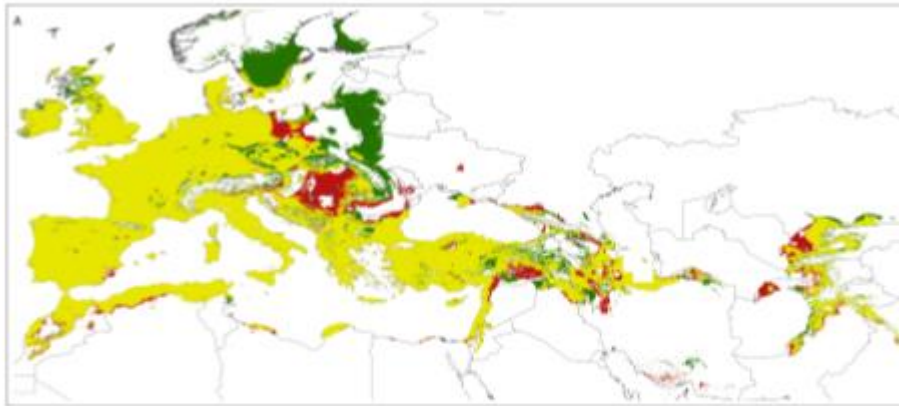
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# Collecting activity on CWR project

Reported by Millenium Seed Bank, Kew

Portugal	Daucus	carota	subsp.	halophilus	4
Portugal and Cyprus	Daucus	carota	subsp.	maximus	6
Portugal and Italy	Daucus	carota	subsp.	maritimus	7
Portugal, Cyprus, Italy, Azerbaijan	Daucus	carota	subsp.	carota	20
Portugal, Italy, Georgia, Azerbaijan and Armenia	Daucus	carota			22
Portugal	Daucus	carota	subsp.	azoricus	5
Portugal	Daucus	carota	subsp.	gummifer	4

In total, 198 accessions from 29 countries – material acquired from 1973 onwards



## *D. carota carota*: potential and future (2050) habitat

Based on CWR work looking at occurrence data (floras, herbaria, genebanks), and environmental parameters along with standard climate change scenario

(Powers, 2014 – MSc thesis)

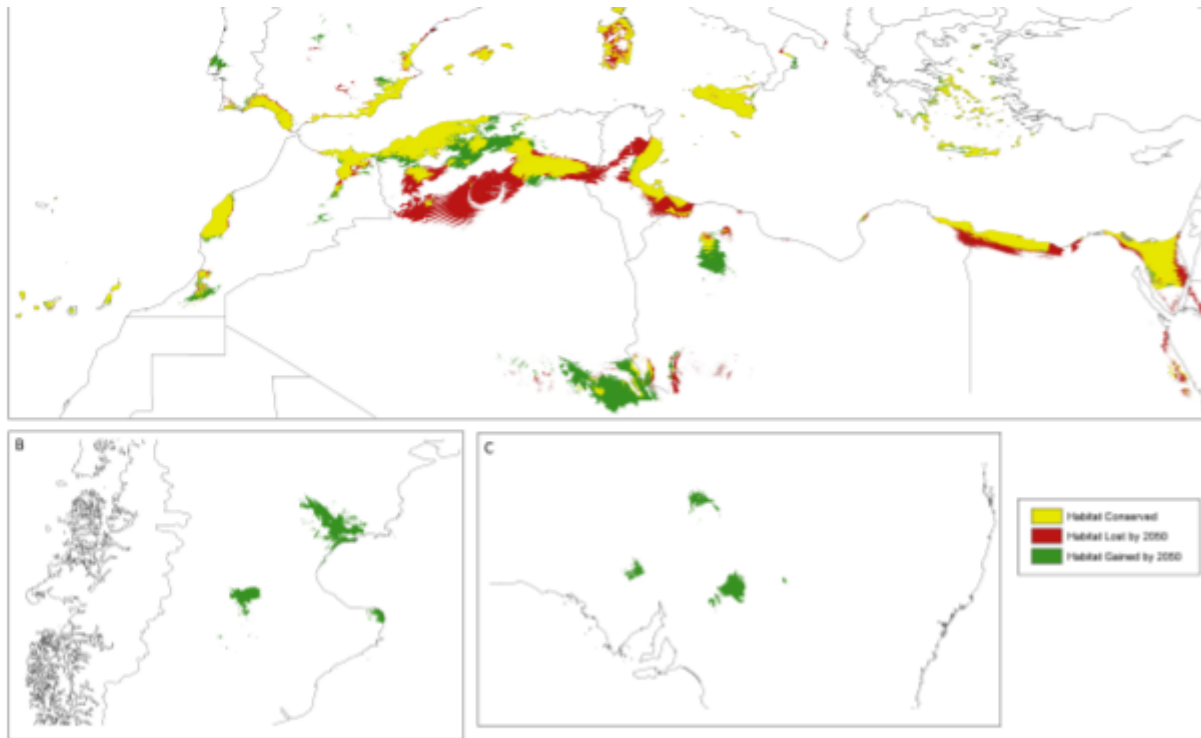


Figure 2: The predicted global range of *Daucus capillifolius* at present and in 2050. A) Mediterranean Region B) South America C) Southern Australia.

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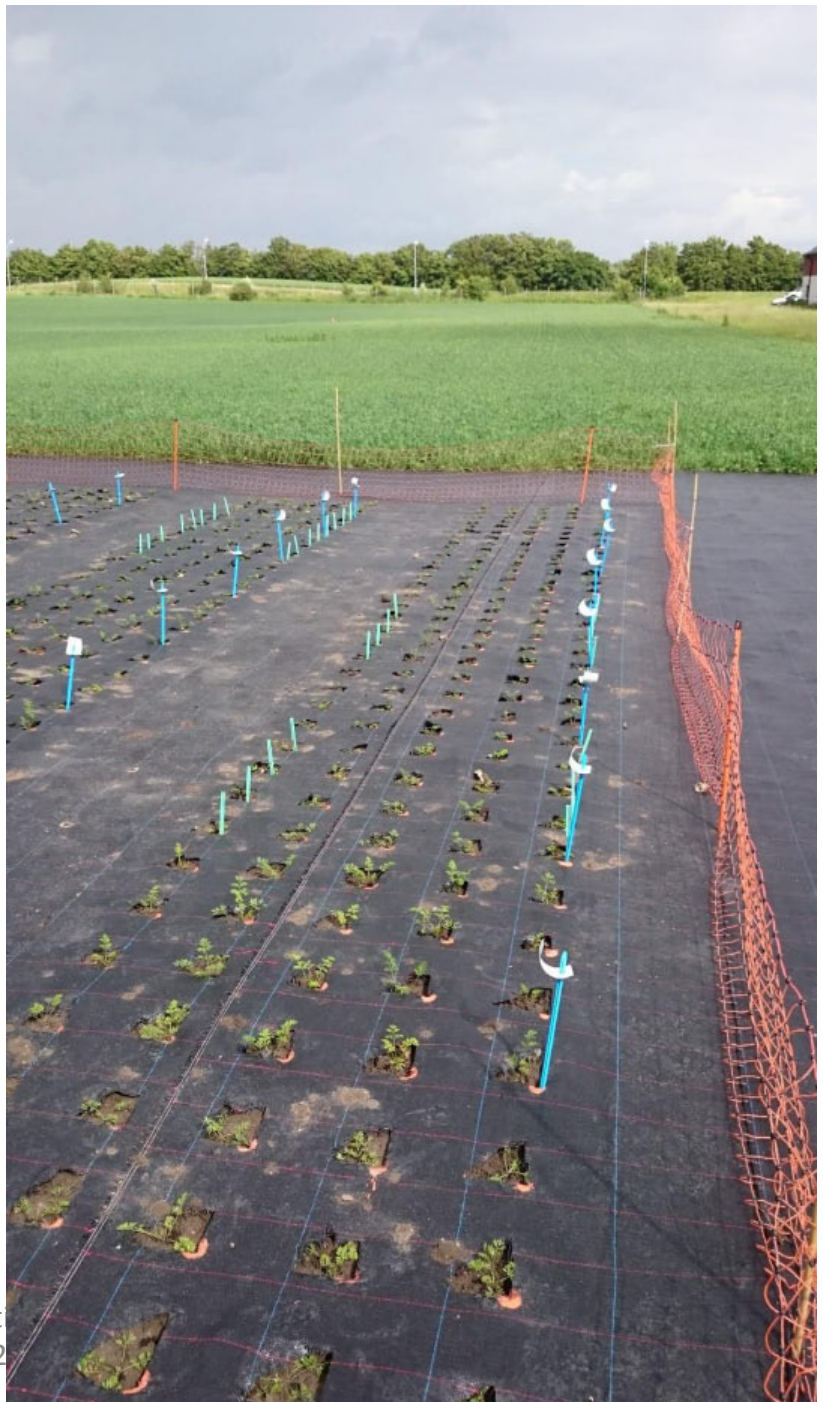
# Global conservation strategy for *Daucus*

- Can we lead/contribute to a strategy?
- Is it necessary?
- What other people/organisations must be involved?
- ECPGR In situ WG....
- What (if anything) is missing from existing projects?
- What don't we know about?
- How should we move forward?

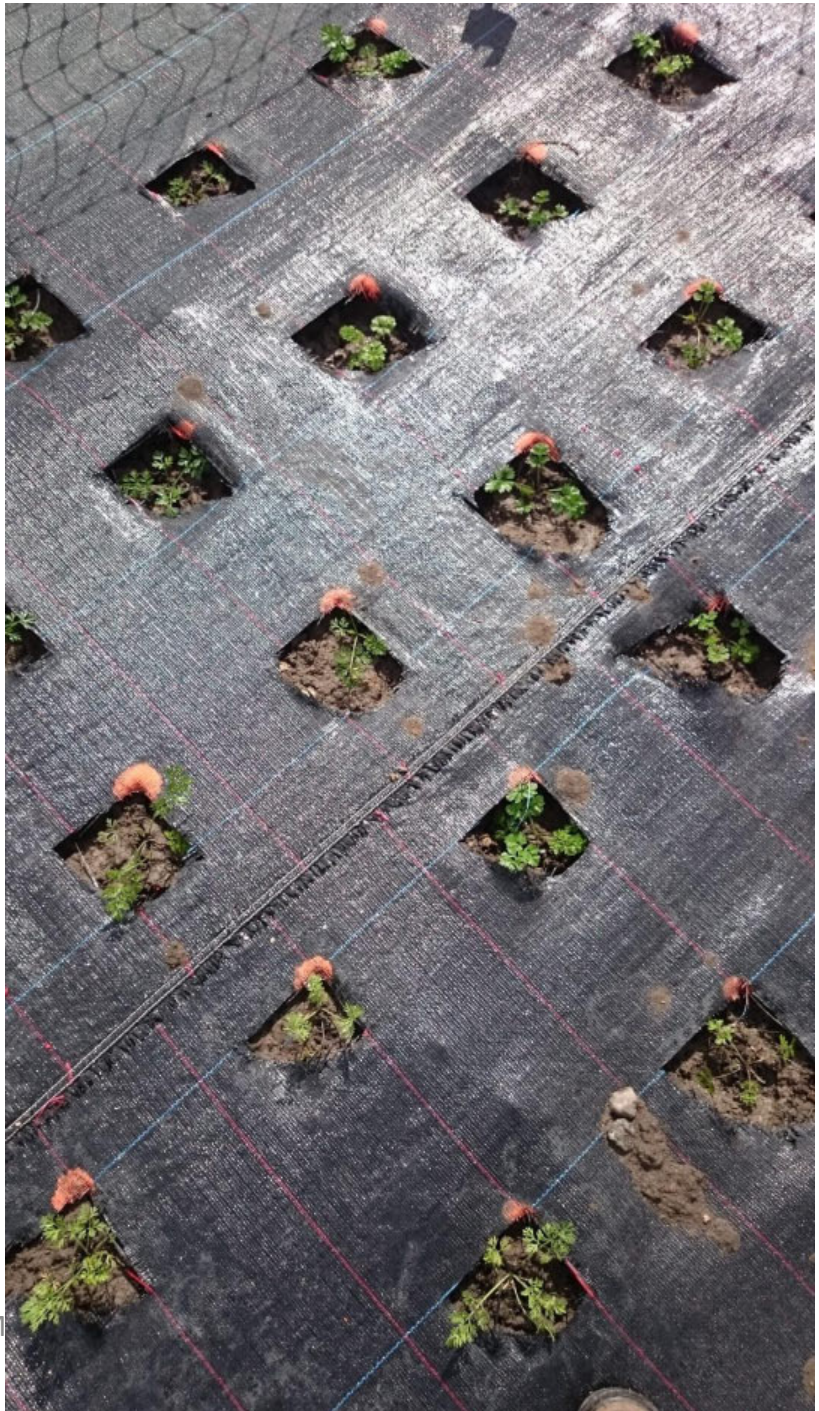
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# CarrotDiverse project protocols and future plans

- Confirmation of sowing/transplantation dates
- Selection and harmonisation of phenotyping methods
  - Traits/descriptors
  - Scoring and digital imaging
- Taxonomic confirmation
- Disease resistance phenotyping
- Genotyping – sampling and protocols







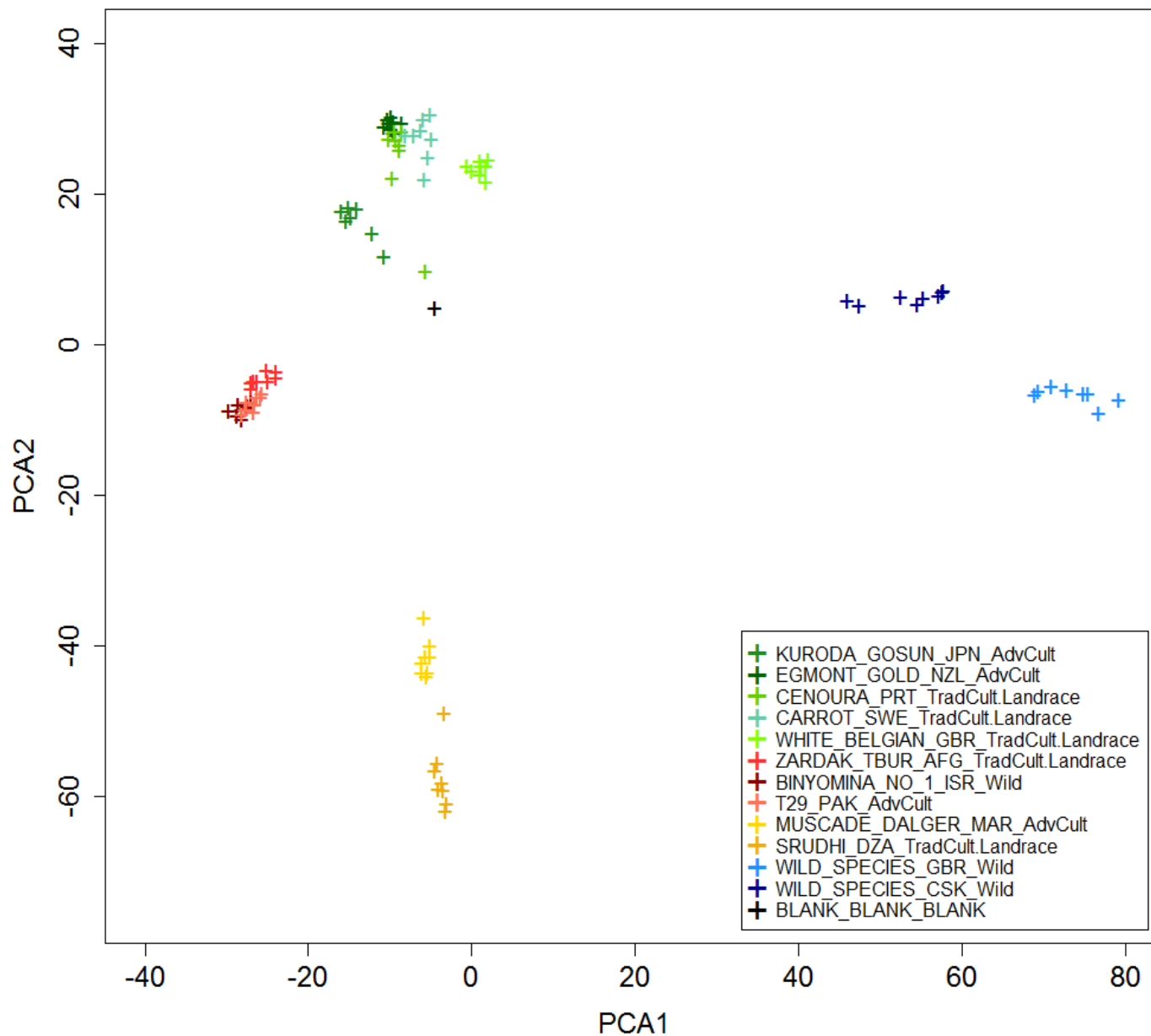
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# Genotyping by sequencing (GBS)

- Short sequence fragments, aligned to find SNPs
- Sampling strategy to be agreed
  - Project enables 96 samples to be genotyped...
  - Association between markers and traits
  - Genepool diversity studies
  - Inter vs intra accession diversity
- DNA extraction requirements (good quality, high MW DNA needed)
- Collation of samples from different partners if necessary



**Figure 1** Plot of the first and second Principal Components taken from a PCA carried out on all SNPs called in the TASSEL pipeline.



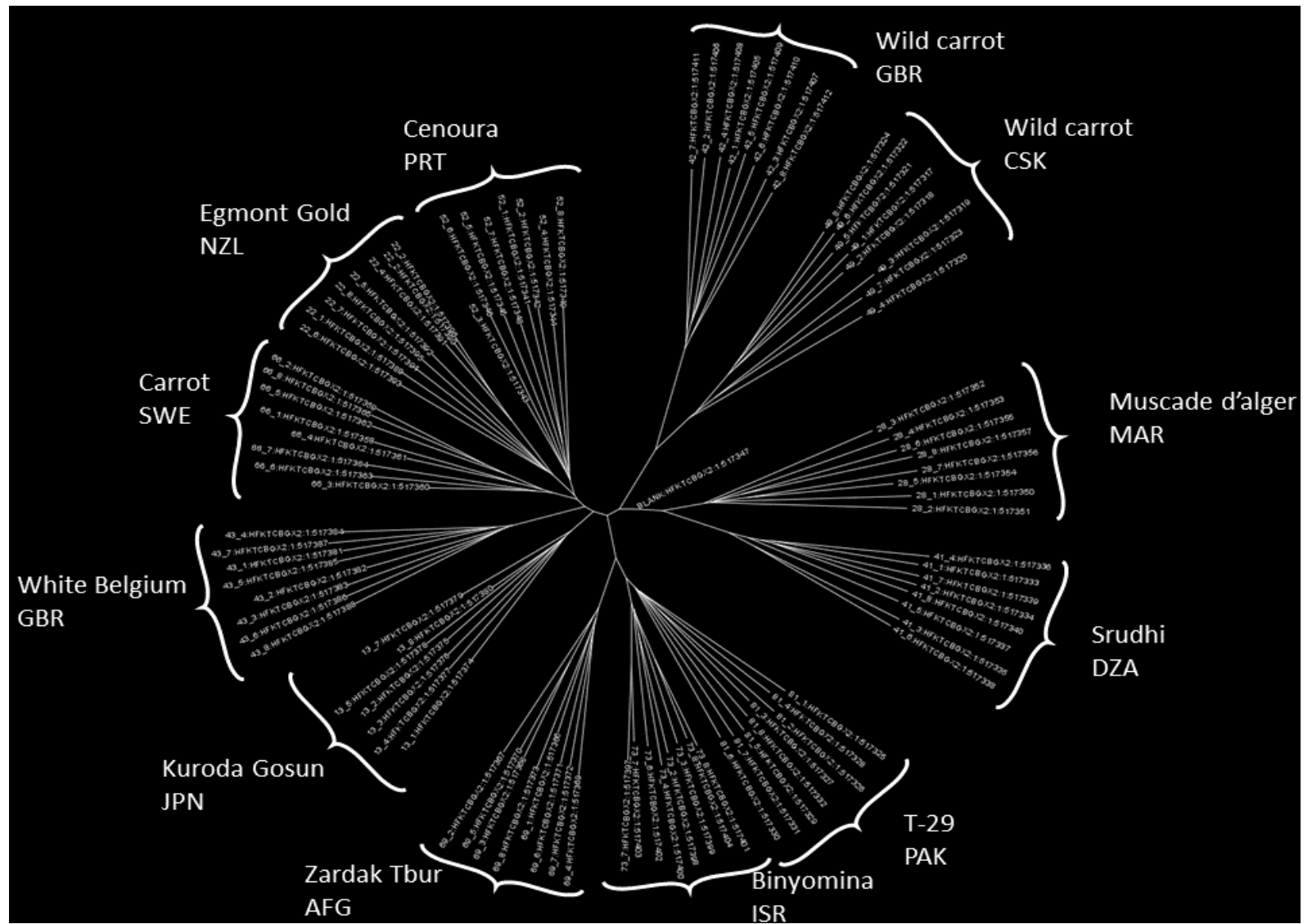


Figure 2 Unrooted phylogenetic tree.