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CarrotDiverse: understanding variation in a wild relative of carrot

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Abstract

Genebanks and other *ex situ* collections have a significant role in the conservation, management and use of crop genetic diversity, including that of crop wild relatives. Efficient management and use depends on insight into the patterning and distribution of genetic diversity as well as obtaining baseline information on phenotypic characters and traits. Wild carrot (*Daucus carota* L.) is the closest wild relative of cultivated carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Arcang.), and is a potential source of useful traits for crop improvement. There are over 900 accessions of *D. carota* described as wild in European genebanks, however associated phenotypic and genotypic characterisation data are sparse. The influence of environment on phenotype is also not well understood in this taxon, meaning that it is difficult to ascertain how data collected at different locations can be compiled and collated. We present initial results of CarrotDiverse, a collaborative project in which wild *D. carota* accessions are undergoing detailed phenotypic and morphological characterisation in parallel at three sites of varying latitude across Europe located in Portugal, France and Sweden. This will allow us to understand which traits are affected by environmental variables. Basic phenotypic information is being collected on a further 150 accessions. Furthermore, resistance screening to *Alternaria* species and polyacetylene profiling will be included in the evaluation. A Genotyping by Sequencing approach will be used to generate knowledge about genetic background and trait associations. The project will result in a significant dataset which will facilitate the use of crop wild relatives in carrot breeding and improvement.

Keywords:*Daucus*, crop wild relative, plant genetic resources, characterisation, genebank

INTRODUCTION

Plant genetic resources for food and agriculture offer the raw material of genetic diversity for crop improvement programmes and underpin scientific investigations into topic such as trait biology and genetics and evolutionary biology and domestication. In total, an estimated 7 million accessions are conserved in genebanks across the world (FAO 2010). Carrot is an economically and nutritionally significant vegetable crop and significant germplasm collections are conserved in a range of institutes globally. Over 6,400 accessions are reported as being conserved worldwide by the Genesys database (www.genesys-pgr.org, accessed 18th May 2018). Over seventy percent of this material is classified as cultivated material or breeding lines, with 1842 accessions being tagged as representing carrot crop wild relatives (CWR). CWR offer a potentially much expanded reservoir of genetic diversity and novel traits as these species and populations contain alleles which have been excluded from the genepool of cultivated material through domestication and intentional or unintentional selection by farmers and breeders. Useful traits sourced from wild species include resistance to carrot root fly (*Psila rosae*) (Ellis et al. 1993) and resistance to fungal pathogens (Nothnagel et al. 2017).

Due to the sheer numbers of accessions conserved, and the decentralised nature of global crop collections, collaborative projects and activities are essential in order to support utilisation by end users and streamline collection management through the identification of unique and significant material. In Europe, the European Cooperative Programme on Plant Genetic Resources (ECPGR) aims to ensure the long term conservation and facilitate the utilisation of plant genetic resources across 33 member countries. The activities of ECPGR revolve around 18 crop focused and three thematic working groups (WG); membership of these groups is comprised of crop experts nominated by ECPGR member countries. One of the groups (the Umbellifer Crops Working Group (www.ecpgr.cgiar.org/working-groups/umbellifer-crops) has responsibility for carrot and other apiaceous crops and has developed collaborative approaches including crop specific genebank standards and a minimum set of descriptors for consideration in phenotypic characterisation trials.

Previous research activity within the Umbellifer Crops Working Group aimed at supporting collection management and utilisation includes taxonomic verification work, screening for resistance to *Alternaria* spp., phenotypic characterisation and investigation of taxonomic differences in genome size through flow cytometry. Detailed phenotypic characterisation of 14 accession was undertaken, including morphological descriptors and flowering behaviour of *Daucus carota* subsp. *carota* (Solberg and Yndgaard 2015), finding that sowing time had a strong influence on flowering time, with earlier sowings resulting in increased annual behaviour. Relative genome size in wild *Daucus* accessions was studied by flow cytometry (Allender 2014) with the finding that *D. carota* accessions of the subspecies *gummifer* had a slightly increased genome size compared to accessions of subspecies *carota*, and genome sizes in other *Daucus* species such as *D. litoralis* and *D. glochidiatus* had much larger genome sizes. Screening for resistance to *Alternaria dauci*, *A. alternata* and *A. radicina* was carried out using 30 accessions of wild *D. carota*, finding eight and ten accessions exhibiting lower disease severity scores than cultivated controls to *A. dauci* and *A. alternata*, respectively (Nothnagel et al. 2017).

The importance of characterising genebank material using a common set of robust descriptors has been highlighted by the previous activities of the Umbellifer Crops WG. Two extensive sets of descriptors have been developed for cultivated carrot (IPGRI 1998), (UPOV 2015). Despite previous efforts, basic morphological data do not exist for many accessions of carrot crop wild relatives in European genebanks, potentially hindering their deployment in crop improvement and research. The project CarrotDiverse was conceived as a way to collaboratively approach this problem, taking advantage of the different geographical locations (most obviously latitude) of project partners to allow an investigation not only the diversity of key morphological traits of carrot crop wild relatives in key European collections, but also to gain a better understanding of which traits are strongly influenced by the trial environment and which are more robust to environmental effects. Beginning in 2017, the project aims to carry out a detailed, replicated phenotypic screen of ten wild accessions of *D. carota* across three sites and over two years. In parallel up to 150 additional accessions will be grown out and will undergo basic morphological characterisation. A subset of material will be further investigated for resistance to *Alternaria* spp, undergo polyacetylene profiling and characterisation of genetic diversity via Genotyping by Sequencing and this work will be reported elsewhere.

METHODS

Ten accessions (Table 1) were selected for detailed replicated characterisation at three sites (Braga, Portugal – 41.5°N; Angers, France - 47.5°N; Alnarp, Sweden - 55.6°N). Seed were sown in the final week of April 2017 into transplant trays and transplanted into the field between 20th and 25th May 2017. The decision to use transplants enabled seed to be sown despite unfavourable field conditions in some of the sites early in the growing season, however this meant that root traits could not be assessed. Three replicate blocks were transplanted at each site, with twenty plants per accession in each block. Phenotypic characterisation was carried out on twelve plants per accession per block. The entire trial will be repeated in 2018 to obtain a further year of data.

Accessions were qualitatively assessed for a number of traits including petiole anthocyanin colouration, homogeneity at rosette stage, rosette leaf growth habit, rosette leaf dissection, petiole hairiness, homogeneity at flowering stage and stem hairiness. Quantitatively assessed traits included petiole thickness, width of the first umbel, total number of umbels at harvest, seed weight, lifespan (days to bolting and flowering as a percentage of total plants in each plot). Traits were scored according to published descriptors for carrot (IPGRI 1998). After collation, data for qualitative traits were subjected to an initial analysis using the Scheirer-Ray-Hare test to look for differences among accessions, taxa and locations. Quantitative data were tested using a two way ANOVA to identify difference attributable to accession, trial location or an interaction between the two. Statistical tests were implemented in R and significance thresholds were set at $p<0.05$.

Table 1. Details of the ten accessions selected for replicated phenotyping screen

Accession ID	**Taxon**	**Country of origin**	**Habitat**	**Latitude**
NGB21386	*D. carota* ssp. *carota*	Norway	unknown	60°N
HRI 8001	*D. carota* ssp. *carota*	UK	continental	52°N
ACO/IRHS 886	*D. carota* ssp. *carota*	France	continental	47°N
BPGV 08159	*D. carota* ssp. *carota*	Portugal	continental	41°N
HRI 8716	*D. carota* ssp. *gummifer*	UK	sea coast (Atlantic)	50°N
ACO/IRHS 710	*D. carota* ssp. *gummifer*	France	sea coast (Atlantic)	43°N
BPGV08432	*D. carota* ssp. *hispidus*	Portugal	sea coast (Atlantic)	37°N
BPGV08391	*D. carota* ssp. *maximus*	Portugal	continental	39°N
ACO/IRHS 715	*D. carota* ssp. *gadecaei*	France	sea coast (Atlantic)	47°N
ACO/IRHS 706	*D. carota* ssp. *maritimus*	France	sea coast (Mediterranean)	43°N

RESULTS

Data collected during the first of the two planned growing seasons revealed a range of relationships between trait, accession, taxon and trial location (Table 2). Traits were regarded to be robust to environmental effects if there was no significant impact of trial environment on trait scores, or if there was no significant interaction effect between accession and environment. The qualitatively scored traits which appeared to be robust to environmental effects were petiole anthocyanin colouration and petiole hairiness. Stem hairiness at the flowering stage in contrast was impacted by trial location. Rosette leaf growth habit and rosette leaf dissection were also impacted by the trial environment.

Some traits such as the number of umbels at harvest (Figure 1) exhibited a clear gradient with latitude, with plants of the same accession grown in Portugal having reduced numbers of umbels than those grown in France or Sweden. Traits such as those related to flowering phenology (time to bolting and flowering measured as a percentage of plants for each accession replicate) also exhibited a relationship with trial location.

Table 2. Assessment of descriptors based on results of 2017 field season. The effectiveness of descriptors is shown at accession and taxon level, and if the descriptor is strongly influenced by the location of the field site. Effective descriptors showed a difference either at accession or taxon level ($p<0.05$).

Life stage	**Descriptor**	**Accession level**	**Taxon level**	**Location effect**
Mature rosette	Petiole anthocyanin colouration	X	X	
	Homogeneity at rosette stage			X
	Rosette leaf growth habit			X
	Rosette leaf dissection			X
	Petiole hairiness	X		
	Petiole thickness		X	
Flowering	Homogeneity at flowering stage			X
	Stem hairiness			X
	Width of the first umbel	X		
	Total number of umbels			X
Fruit	Fruit weight	X		
General	Lifespan (% bolting/flowering)			X

DISCUSSION

The results are based on the first of the two growing seasons, and may obviously be impacted by the trait patterns exhibited during the 2018 growing season. As such no firm conclusions can therefore be drawn at this stage however it is possible to discuss the outcomes of the 2017 trial in the context of previous work in this area.

Solberg and Yndgaard (2015) looked at morphological characters as well as flowering time in a set of wild *D. carota* collected in Sweden, Denmark and Norway. They found that here was both inter- and intra-accession variation in many traits, with weak geographic clustering but no clear effects of latitude. They also noted the impact of sowing time on flowering behaviour, with earlier sowings resulting in higher levels of an annual growth habit,

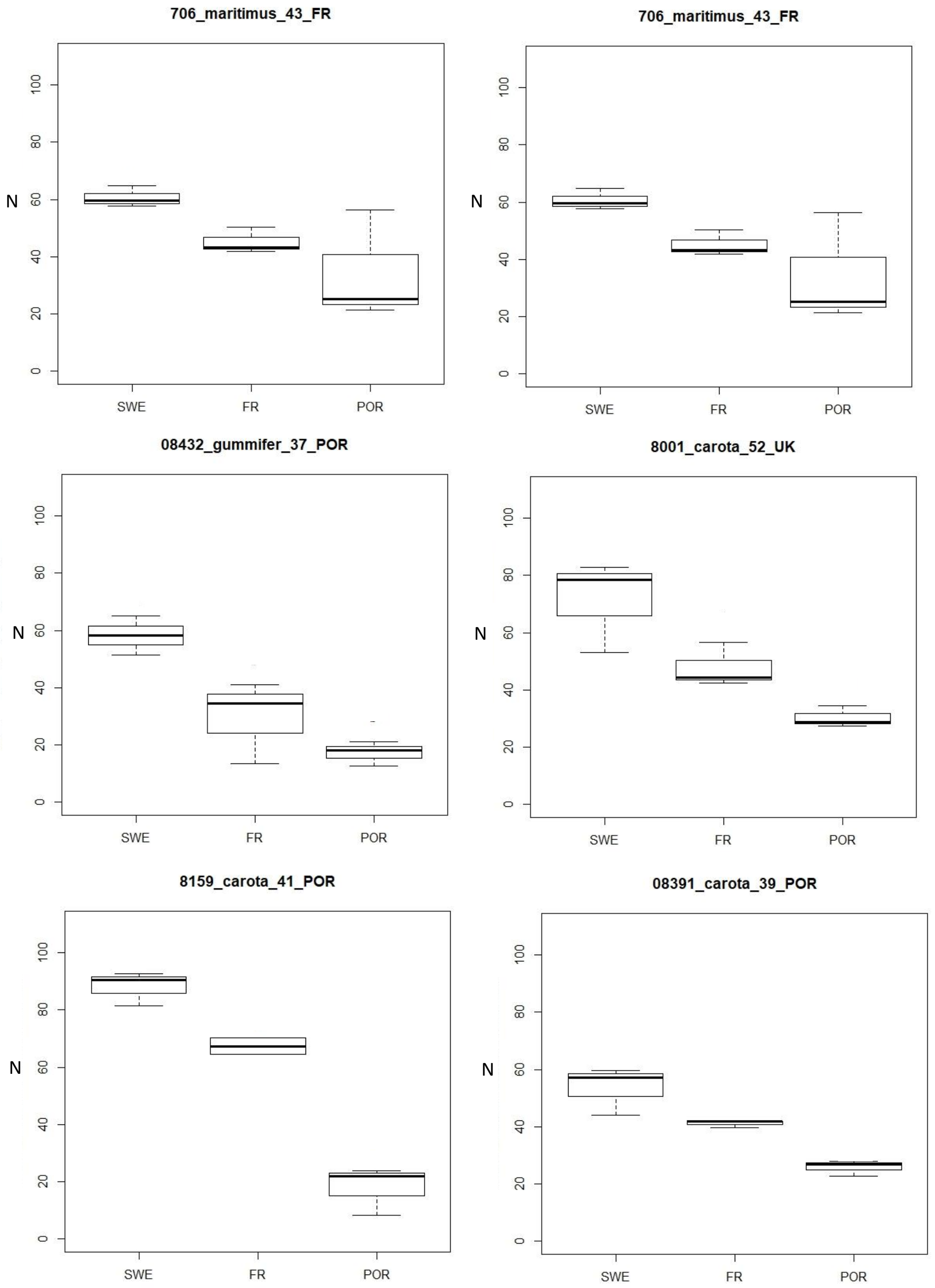

Figure 1. Total numbers of umbels at harvest (N) for six accessions. Data indicate a strong and predictable influence of environment with a gradient from Sweden to Portugal. Other accessions showed a similar pattern (data not shown). Title of each panel indicates accession identifier, taxon, latitude of original collection site and country of collection. Trial locations are SWE – Sweden, FR – France and POR – Portugal

but this effect varied in the two years of the experiment. Differences in the temperature of the early part of the growing season was identified as a possible cause. Our data confirm the plasticity some aspects of morphological phenotype; stem hairiness and total number of umbels at harvest are clear examples of this. Other traits potentially affected by environment include bolting/flowering behaviour, leaf growth habit and leaf dissection. Additional data from the 2018 field trail will help to confirm the degree of environmental influence on these traits. Populations of *D. carota* and related taxa are known to be quite variable; other studies have found a relatively high degree of variation in morphological character states among accessions of *D. carota* and subtaxa; see for example (Spooner et al. 2014) and (Arbizu et al. 2014).

The second year trials are well under way and will yield data from a further growing season, enabling further determination of the influence of environment on the phenotypic traits scored, and together with the meteorological data from each trial site will enable further study of the impact of environmental conditions on morphology and flowering.

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