Morphological and phenological diversity in Scandinavian wild carrot

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Abstract

Wild carrot is a valuable source of genes for crop improvement but numerous undesired traits accompany wild species. First year flowering (bolting) is one such trait. A study was carried out to investigate Scandinavian wild carrot accessions. Plants were seeded in early spring or in summer. Early seeding resulted in bolting in the wild accessions, where more than 20% of the plants produced seeds during the first year. The result demonstrates the partly annual growth habit of wild carrot. All accessions were classified as *Daucus carota* subsp. *carota*. Morphological variations among and within accessions were detected but clear geographical clustering was not found. The results are discussed with emphasis on

conservation and utilization of crop wild relatives and how within-accession diversity can be a challenge for established *ex situ* conservation system.

Key words

Bolting; Conservation; Crop wild relative; *Daucus carota*; *Ex situ*; Genebank management; Population; Regeneration

Introduction

Crop research is mostly undertaken with cultivars; however, to sustain productivity there is a constant need for new genes (Swanson, 1996; McCouch et al., 2013). Due to its wide adaptation, wild carrot (*Daucus spp.*) could be a valuable potential source of genes that are of interest, including resistance and tolerance to adverse biotic and abiotic factors, as well as male sterility (GenRes, 2002; Camadro et al., 2008; Nothnagel et al., 2014). However, as wild crop relatives have a substantial number of undesired traits, working with crop wild relatives in breeding is fraught with complicating factors. In cultivated carrot (*Daucus carota* L. var. *sativus* Hoffm., 2*n* = 18), one of the most obvious undesired traits is bolting. Bolting is premature plant flowering, induced by low temperature and long day conditions in biennial plants (Heide, 1973; Guttormsen and Moe, 1985; Ritz et al., 2010).

Carrot is one of the major vegetables worldwide (FAOSTAT, 2015). Wild carrot originates in the Mediterranean region, particularly North Africa, and was first cultivated in Central Asia. Studies have led to the recognition of around 20 *Daucus* species worldwide, of which 15 are found in the Mediterranean region (Sáenz Laín; 1981; Rubatzky et al., 1999). However, the *Daucus* taxonomy has been and continues to be subject to debate (Small, 1978; Zohari, 1987; Spooner et al., 2014). In Scandinavia (Sweden, Denmark and Norway), *Daucus carota* is the only species, with the subspecies *carota* being by far most the most common (GBIF, 2014) (Table 1). The morphological support for subspecies classification is questioned by

Spooner et al. (2014), who concluded that in addition to cultivated carrot there are most likely only two subspecies of *D. carota*; subsp. *carota* and subsp. *gummifer.*

Genebanks have been collecting and conserving plant genetic resources, including crop wild relatives, for decades; however, the number of accessions of *Daucus* held in germplasm collections around the world is relatively small and consists of approx. 5,600 accessions globally. Important collections are held at the Vavilov Institute in Russia, at Warwick University in the UK and at the US Department of Agriculture (Rubatzky et al., 1999). The collection at the Nordic Genetic Resource Center in Sweden has 182 cultivated and 18 wild carrot accessions.

Table 1. Occurrences of different subspecies of *Daucus carota* according to GBIF (2014).

Taxon	Global Occurrences	Scandinavia Geo-referenced occurrences
Daucus carota L.	124942 [a]	6946
Daucus carota ssp. *carota*	122297 [a]	6943
Daucus carota ssp. *gummifer* Hook	1,161	1[b]
Daucus carota ssp. *azoricus* Franco	28	0
Daucus carota ssp. boissieri.Hosni	12	0
Daucus carota ssp. cantabricus Pujadas	13	0
Daucus carota ssp. commutatus Thell.	83	0
Daucus carota ssp. drepanensis Heywood	17	0
Daucus carota ssp. gadecaei Heywood	20	0
Daucus carota ssp. halophilus Pujadas	20	0
Daucus carota ssp. hispanicus Thell.	233	0
Daucus carota ssp. major Arcang.	30	0
Daucus carota ssp. majoricus Pujadas	30	0
Daucus carota ssp. maritimus Batt.	285	0
Daucus carota ssp. maximus Ball.	713	(2)[c]

[a] Occurrence records of *Daucus carota* ssp. *carota* are not provided by GBIF but calculated on the basis of the numbers of *Daucus carota* L. occurrences minus the occurrences of the above given subspecies other than subsp. *carota*.
[b] The only geo-referenced record is from a small island between Sweden and Denmark
[c] Geo-referenced specimen from Gothenburg, probably not wild, but material transferred to the area.

A common challenge for collection holders is the lack of characterization and evaluation data on the germplasm. Without such data the genetic material is not readily available for users. The international community therefore regards characterization and evaluation as an important objective. The present study examines the wild carrot collection at the Nordic Genetic Resource Center, emphasizing morphological and phenological traits. The main objectives were to verify taxonomical classification, to obtain an understanding of diversity among and within accessions and to detect inter-trait relationships. The main hypothesis was that accessions from a relatively limited area in Scandinavia show similar traits. The result is relevant for prospective collection missions in the region. Furthermore, knowledge on the diversity among and within accessions is relevant for regeneration procedures. Taxonomy as well as characterization results are relevant for genebank management and communication and distribution of material for breeding and research.

Table 2. Passport data of the examined accessions [a]

Accession number	Accession name	Region and country	Geo-reference	Altitude
NGB21387	Lomma SS080904	Skåne, Sweden (SE)	55°41’N, 013°06’E	13
NGB21388	Alnarp SS080904	Skåne, Sweden (SE)	55°39’N, 013°05’E	10
NGB21389	Klostergården SS0101	Skåne, Sweden (SE)	55°41’N, 013°10’E	21
NGB16882	Lerhamn KO2005	Skåne, Sweden (SE)	56°15’N, 012°31’E	18
NGB20104	Röttle AB080925	Småland, Sweden (SE)	58°01’N, 014°26’E	110
NGB21956	SS0809110101	Skåne, Sweden (SE)	55°36’N, 013°03’E	10
NGB21386	Oscarsborg SS0909	Akershus, Norway (NO)	59°40’N, 010°36’E	10
NGB21129	Gisseløre GP0102	Sjælland, Denmark (DK)	55°40’N, 011°04’E	1
NGB21705	Agersø SS0704	Sjælland, Denmark (DK)	55°12’N, 011°11’E	5
NGB21720	Sjællands odde SS0102	Sjælland, Denmark (DK)	55°57’N, 011°27’E	2
NGB21878	Rønne SS20091008	Bornholm, Denmark (DK)	55°05’N, 014°41’E	2
NGB21924	Borreby SS20090921	Sjælland, Denmark (DK)	55°13’N, 011°17’E	5
NGB23506	Kollerup KW0710230101	Jylland, Denmark (DK)	56°19’N, 010°03’E	63
NGB24386	Vestborg fyr SS20120926	Jylland, Denmark (DK)	55°46’N, 010°33’E	5
NGB547	Nantes Asso	Commercial cultivar, included as reference		

[a] More information is available from the germplasm database SESTO (2015).

Material and methods

Plant material and characterization

The study included 14 wild accessions from different locations in Scandinavia, in addition to one commercial cultivar that was used as a reference (Table 2). Ten plants per accession and treatment were examined. Plants were seeded and thinned to one plant per pot in a 20 cm deep pot with an upper diameter of 5 cm. Three seeding time treatments were used; 1) seeding 3 March 2013, 2) seeding 1 July 2013 and 3) seeding 12 March 2014. The soil was a moderately pre-fertilized soil (S-JORD from Hasselfors garden, Örebro, Sweden). After seeding, the plants were placed in a cold greenhouse, allowing fluctuations in temperature due to outside temperature but with a frost protection system starting heating when temperature drops down to 8-10° C. After 60 days in the greenhouse the pots were moved outside and placed in sand bed. Plants were irrigated twice a day during the experimental period.

Table 3. Descriptors with explanations.

Explanatio
Leaf characters (scored 60-80 days after seeding)
Leaf length; measured in cm
Leaf green colour; visual score; 3=light, 5=medium, 7=dark
Number of leaflets; counted per leaf
Leaf dissection; visual score; 3=slightly, 5=intermediate, 7=highly
Leaf growth habit; visual score; 3=prostrate, 5=semi-erect, 7=erect
Petiole anthocyanin colouration; scored yes or no after 60 days (in % of the plants)
Petiole hair; visual score; 3=few, 5=medium, 7=many
Petiole thickness (mm)
Petiole shape, 1=round, 2=semi-round, 3=flat
Stem characters (scored 120-150 days after seeding)
Stem length (cm)
Stem colour; visual score; 2=green, 4=purple green
Stem hair; visual score; 3=few, 5=medium, 7=many
Stem growth habit; 3=prostrate, 5=intermediate, 7=erect
Branches per plant; counted at flowering
Stem leaves, below primary umbel; counted per plant
Flower characters (scored 100-150 days after seeding)
Flower colour; visual score; 1 is white, 4 pink, 5 purple, 15 is mixture
Primary umbel size; width measured in cm
Umbellets' size; diameter measured in mm
Number of umbellets; counted in primary umbel
Umbel shape; visual score at full blooming; 1= convex (nest-like), 2= flat, 3= concave
Early bolting; scored yes or no, 100 days after seeding (in % of the plants)
Medium bolting; scored yes or no, 125 days after seeding (in % of the plants)
Late bolting; scored yes or no, 150 days after seeding (in % of the plants)

The morphological and phenological scoring was made at various times using the IPGRI descriptors list for wild and cultivated carrot, but with the use of SI units (IPGRI, 1998) (Table 3). The descriptors were selected as they represent an international standard and are regarded as important for breeding and for the maintenance of germplasm collections. One scoring was made for each of the 10 plants per accession and treatment. Details on scoring method and time for scoring are given in Table 3.

Analytical methods

Statistical analysis was done using R software (R Core Team, 2014). The eight most completely scored descriptors were; leaf length, leaf growth habit, number of leaflets, leaf dissection, early bolting, medium bolting, late bolting and petiole anthocyanin colouration. These were selected for the further analysis. The R function *boxplot* was used to illustrate the distributions of the different descriptors. In order to obtain an overview of the material, a principal component analysis was performed using the R function *princomp*. This was done based on each accession's mean values. A scatterplot matrix was set up to describe the relationship among descriptors (Everitt and Hothorn, 2011). Genebanks often use the UPOV system with categorical scales. For example, we scored the green colour similarly using a low value (3) for light, a medium value (5) for intermediate and a high value (7) for dark. A scatterplot matrix describes the pairwise relationships among all descriptors in a very useful way. The upper right triangle illustrates the scatterplots while the lower left triangle shows the calculated correlation coefficients. The histograms are shown on the diagonal. Since the correlation must be interpreted with caution, the locally weighted scatterplot smoother is drawn. Standard ANOVA tests using the R function *aov* were used to identify significant differences in means. Significant descriptors were analysed further using Tukey’s multiple comparisons of means (Crawley, 2009) with a 95% family-wise confidence level to identify the differences at the 5% level. We tried to apply imputed values but realized that for some descriptors, too many values were missing. Instead, we selected the descriptors that lacked less than 20% of their values for the analysis. Temperature records were downloaded from the closest meteorological station at Malmö (SMHI, 2015).

Results

Overall patterns

On average, all the wild accessions showed a prostrate growth habit and had petiole hairs (Table 4, Table 5). All plants from the wild accessions had white root colour. The biplot from the PCA analysis is presented with the eight most complete characters and without the reference cultivar (Figure 1). This is useful for interpretation of the variability of the wild material. Here, the two first variance components explain 55% of the total variation (30% and 25%, respectively). Only some clustering based on country of origin could be detected, but no effect of latitude or altitude could be found.

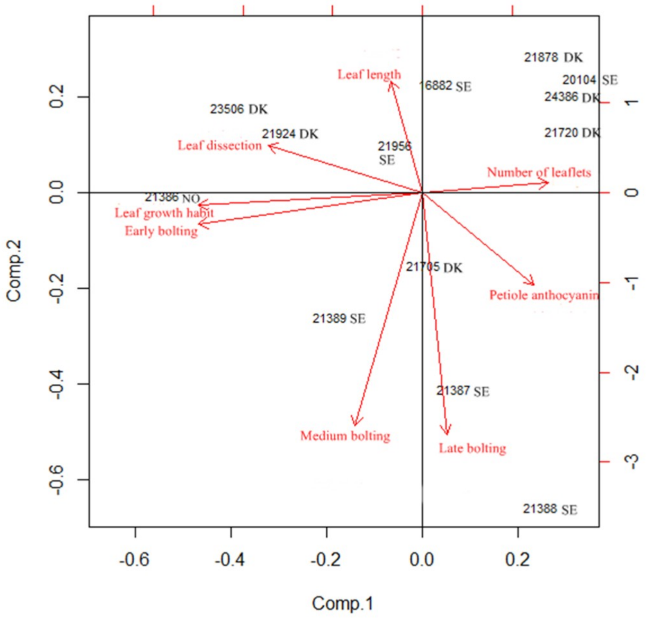

Figure 1. PCA biplot based of mean values of each accession. The commercial cultivar and NGB21129 have been removed from the analysis.

Flowering in the year of seeding (bolting)

Seeding time greatly influenced bolting, with seeding in July resulted in no bolting in any of the accessions, and seeding in March resulting in bolting in the wild accessions. Different results were obtained in the different years: when seeded in early March, all plants in the wild accessions were flowering in the year of seeding in 2013. In 2014, more than half of the accessions showed some plants with flowers in the year of seeding. The average bolting rate within all wild accessions was 21%. Despite no significant difference, variation was detected among accessions (Figure 2). The figure illustrates bolting as boxplots based on the results after all the three seeding times. A boxplots is a graphical display that consists of a box defined by the 25th and 75th percentiles and a line at the median (50th percentile). The three accessions from Southern Sweden (NGB21387, NGB21388 and NGB21289) showed the highest percentage of bolting plants, followed by two of the accessions from Sjælland, Denmark (NGB21129 and NGB21705).

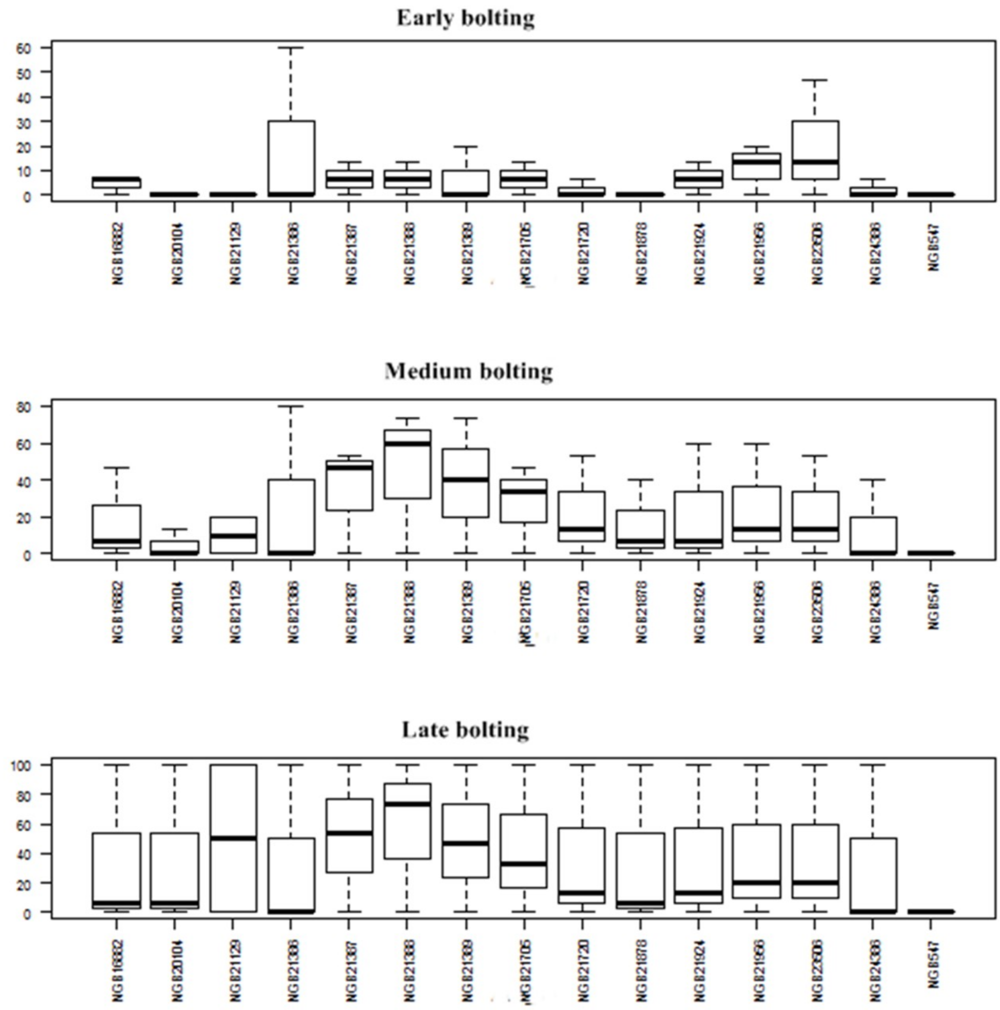

Figure 2. Boxplot of bolting results in the wild carrot accessions (see text for explanation).

Table 4. Mean values and standard deviation for the characters most completely described

Acc_no	Leaf length after 60-80 days (cm)	Leaf Attitude (scale 3-7)	Number of leaflets per leaf	Leaf Dissection (scale 3-7)	Petiole Anthocyanin coloration (%)	Early bolting (%)	Medium bolting (%)	Late bolting (%)
NGB16882	31.7 ± 4.6	3.9 ± 0.7	11.0 ± 1.6	5.4 ± 0.0	27 ± 28	5 ± 4	18 ± 25	36 ± 56
NGB20104	27.7 ± 3.5	3.8 ± 0.6	11.4 ± 2.3	5.2 ± 1.3	47 ± 47	0 ± 0	4 ± 8	36 ± 56
NGB21129	25.1 ± na	4.3 ± na	9.1 ± na	No data	7 ± na	0 ± 0	10 ± 14	50 ± 71
NGB21386	25.6 ± 1.9	4.4 ± 0.4	9.4 ± na	5.2 ± na	7 ± na	20 ± 35	27 ± 46	33 ± 58
NGB21387	26.3 ± 2.5	4.0 ± 0.7	11.4 ± 1.0	5.3 ± 0.9	38 ± 53	7 ± 7	33 ± 29	51 ± 50
NGB21388	28.1 ± 2.1	3.8 ± 0.7	11.9 ± 2.2	4.5 ± 0.5	44 ± 44	7 ± 7	44 ± 39	58 ± 52
NGB21389	26.4 ± 3.8	4.0 ± 0.5	11.7 ± 0.9	6.5 ± 0.2	0 ± 0	7 ± 12	38 ± 37	49 ± 50
NGB21705	25.6 ± 1.6	3.9 ± 0.8	11.0 ± 1.8	5.4 ± 0.8	12 ± 7	7 ± 7	27 ± 24	44 ± 51
NGB21720	27.8 ± 2.6	3.5 ± 0.2	12.2 ± 0.6	5.1 ± 0.4	0 ± 0	2 ± 4	22 ± 28	38 ± 54
NGB21878	30.0 ± 3.7	3.6 ± 0.4	11.9 ± 0.3	5.5 ± 0.6	10 ± 14	0 ± 0	16 ± 21	36 ± 56
NGB21924	28.3 ± 6.6	4.4 ± 0.6	11.1 ± 1.1	5.8 ± 0.8	0 ± 0	7 ± 7	22 ± 33	38 ± 54
NGB21956	28.8 ± 6.2	3.7 ± 0.4	12.3 ± 0.8	6.1 ± 0.3	0 ± 0	11 ± 10	24 ± 31	40 ± 53
NGB23506	33.9 ± 6.1	4.2 ± 1.0	12.2 ± 1.1	6.3 ± 0.0	16 ± 6	20 ± 24	22 ± 28	40 ± 53
NGB24386	26.7 ± 2.2	3.8 ± 0.5	11.7 ± 2.2	4.3 ± 0.0	13 ± 18	2 ± 4	13 ± 23	33 ± 58
NGB547	31.6 ± 3.2	7.0 ± 0.0	9.8 ± 3.0	4.8 ± 0.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 5. Scores of the descriptors that were examined the first seeding time only (average values are given except for categorical data where median values are given and with other types in parenthesis, no observation is indicated as -).

	Petiole thickness (mm)	Petiole shape (category)	Petiole hair (category)	Stem length (cm)	Stem colour (category)	Stem hair (category)	Stem growth habit	Branches per plant (#)	Stem leaves per plant	# of umbellets	Umbellets' size (mm)	Umbel shape (category)	Flower colour (category)	Primary umbel size (mm)
NGB16882	1.8	2(3)	7(5)	-	4	5(3)	7(5)	2	4	28	15.2	1 (2, 3)	15	-
NGB20104	1.7	3(2)	5(7)	64	4	7(5)	7(5)	2	6	33	11.8	1 (2)	15	74
NGB21129	1.9	2(3)	7(5)	53	2	3(5)	5(7)	1	5	36	15.2	2 (3)	15	74
NGB21924	1.9	2(1,3)	7(5)	67	4	5(3)	7(5)	1	5	29	11.5	1 (2)	1	78
NGB21386	2.0	2(3)	5(7)	79	4	5(3)	5(7)	1	5	37	15.2	1 (2, 3)	15	87
NGB21387	2.0	2(3)	5(3,7)	36	2	3(5)	5(7)	2	3	26	12.8	2 (1)	1	64
NGB21388	2.3	2(3)	5(3,7)	60	2	7(5)	7(5)	2	4	35	12.3	1, 2 (3)	15	83
NGB21389	1.8	3(2)	3 (5)	78	2	3(5)	7(5)	1	4	41	11.0	2 (1, 3)	1	73
NGB21705	1.8	3(2)	7(3,5)	62	2	5(3)	7(5)	2	6	35	16.5	1 (2, 3)	1	73
NGB21720	1.8	2(3)	7(5)	34	2	5(7)	7(5)	2	5	29	13.8	3 (1, 2)	1	72
NGB21878	2.1	2(3)	5(7)	35	2	3(5)	7(5)	2	3	26	11.8	2 (1, 3)	1	82
NGB21956	1.9	2(3)	7(5)	58	2	5(7)	7(5)	2	4	25	12.8	1 (3)	1	85
NGB23506	1.9	2(3)	5(7)	74	4	7(5)	7(5)	1	5	29	14.6	2, 3 (1)	1	78
NGB24386	2.0	2(3)	7(5)	33	2	3(5)	7(5)	1	5	26	11.8	1 (2)	1	73
NGB547	2.3	2	3	-	-	-	-	-	-	-	-	-	-	-

Relationship among traits

Based on mean values across all accessions, correlation coefficients significantly different from zero were detected among a few of the traits (Figure 3). Although leaf growth habit showed a negative correlation to number of leaflets per leaf and to late bolting, the scatterplot illustrated that the relationship was not so clear. Several of the descriptors did not follow a normal distribution, which also made their correlation coefficients meaningless.

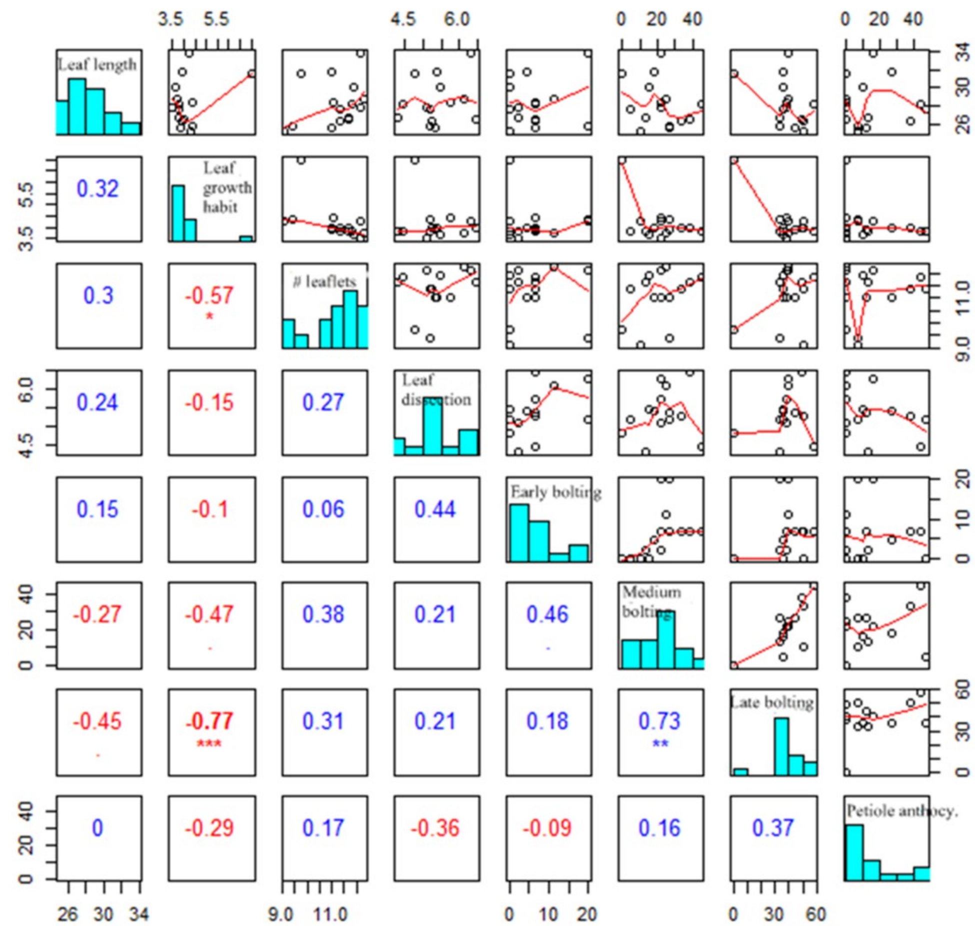

Figure 3. Scatterplot matrix based on mean values across all accessions.

Discussion

Accessions and ex situ conservation

Even though the wild accessions in this study were collected within a relatively limited geographical area in Scandinavia, they display some variation in several of the measured characteristics. However, differences among accessions were not significant due to the considerable variability within accessions. The need of new collection mission within the represented area could therefore be questioned, or rather discussed, in relation to how to maintain diversity within accessions in *ex situ* conservation systems. In the development of conservation strategies, morphological and phenological differentiation must be considered. On one hand, the number of accessions that require collecting and conservation should be high enough to capture the existing diversity. On the other hand, as the material is so diverse, it challenges standard *ex situ* regeneration and maintenance systems (FAO, 2014). A diverse population need high number of plants to capture the diversity both during collection mission and regeneration. (Ellstrand and Elam, 1993; Frankham, 1996; Dittbrenner et al., 2005; Hensen and Oberpieler, 2005; van Treuren et al., 1991). Crossa et al. (1993) point at a need of 160 to 210 plants in wild population to maintain diversity. Our result showed that some plants are annual and some biennial, a finding that challenge the *ex situ* regeneration procedure for wild carrot. Overall, to minimize genetic drift, selection and external gene flow, recollection seems to be a better strategy than regeneration for species and populations that are not threatened, as also suggested by Brown et al. (1997). Furthermore, the within-accession diversity challenges the genebanks' information system, where accessions' traits usually are expressed as one number or one character. In wild accession there is a need to express the range of diversity.

Overlapping collects or duplicates should be avoided in *ex situ* conservation. An example of overlapping accessions in our study was the three accessions from a limited area in Sweden (NGB21387, NGB21388 and NGB21389) that also were close in the PCA plot (Figure 1). From a management perspective the question will be: how to capture as much diversity as possible with the available economic resources?

Diversity and taxonomy

There was a great variability within accessions. Substantial morphologic variability has been previously noted in wild carrot accessions (Camadro et al., 2007; Ibanez et al., 2014; Arbizu et al., 2014) as well as in other crop wild relatives (Song and Walton, 1975; Berger et al., 2002; Pecetti et al., 2008). High diversity also makes it difficult to separate the various subspecies of *D. carota* in morphological terms (Arbizu et al., 2014). In the key to *Daucus*, Sáenz Laín (1981) for example used the shape of the bract and stem length. The work was based on herbarium specimens, which could not include the morphological variation of natural populations. In the descriptor list (IPGRI 1998), the shape of bracts is divided into three categories, but we could observe at least 20 different shapes (illustration not shown). This illustrates the complexity of plant morphology, but also the great diversity found in crop wild relatives. The differences in characteristics, such as stem length and umbel diameter are within the range of what is described for *D. carota* subsp. *carota* (Sáenz Laín, 1981; Spooner et al. 2014). A few of our accessions, however, have a stem that is very short and could be in the range of what is described for *D. carota* subsp. *gummifer* (ibid); but they display a shape of the umbel not typical for subsp. *gummifer*. We therefore conclude that all the wild accessions in the present study are of subsp. *carota*.

Traits and breeding

Some traits scored in our study could be of importance for breeding programmes. Leaf hair is known to protect plants from biotic and abiotic stress (Karabourniotis et al., 1993; Roy et al., 1999). However, traits such as bolting, is unwanted in commercial cultivars. Carrot, or at least cultivated carrot, is believed to be biennial. As mentioned, we could detect flowering in the year of seeding. This could be one way of ensuring reproduction in wild plants. Flowering in wild carrot in the year of seeding has been previously reported by Small (1978). We could detect variation among years. The highest number was found in the year with the lowest air temperature in spring. Figure 4 illustrates that the air temperature in March and April in 2013 was 5-10 ° C lower than in 2014. In both years plants were seeded in early March, with germination occurring within a week. The experiments were carried out in a cold greenhouses with an inside temperature partly dependent on external temperature, albeit with frost protection. Wild carrot seems to be induced at a very early stage or at intermediate temperatures (10-15 °C).

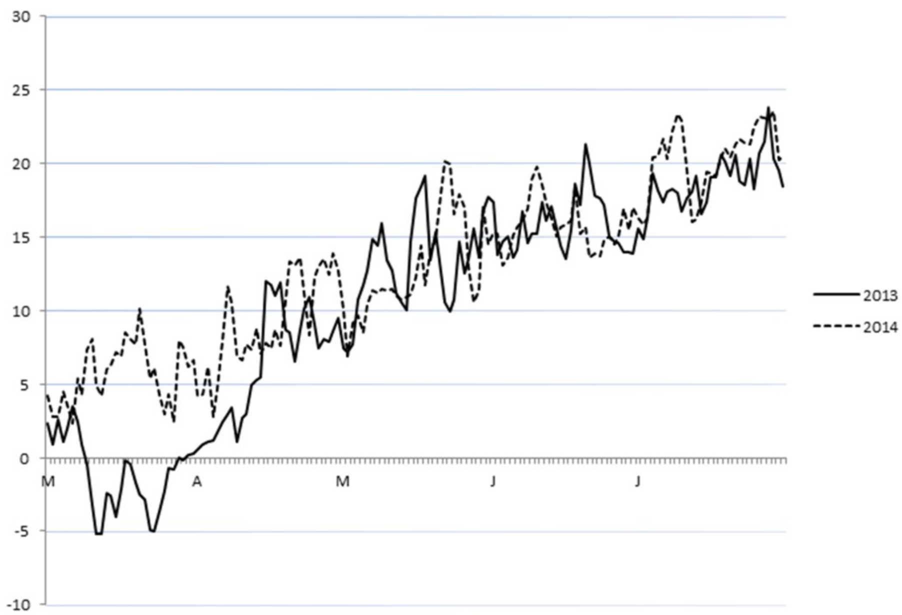

Figure 4. Average 24-hour air temperature from 1 March to end of July in 2013 and 2014.

Conclusion

Our study demonstrated great morphological and phenological within-accessions diversity in wild carrot. Such diversity does challenge existing *ex situ* conservation system. To capture the diversity, samples should be collected from a high number of plants, both at collection mission and at *ex situ* regeneration. Furthermore, the combined annual and biennial nature makes *ex situ* regeneration a challenge and recollection is suggested rather than regeneration of such accessions.

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