

Characterization and distribution of *Daucus* species in Syria

Bassam AL-SAFADI

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria; e-mail: bsafadi@aec.org.sy.

Abstract: Syria is considered as one of the important centres for *Daucus* diversity including *Daucus carota*. Therefore, it is essential to study the distribution and characterization of these species in Syria. An exploration of plants belonging to the Apiaceae family was conducted on road and field sides in several areas of Syria. Seeds (fruits) from these plants have been also collected. The seeds were sown in pots containing peatmoss in a glasshouse and emerging plants were grown until flowering and seed formation. The plants were classified based on leaf, umbel, and seed shape. Proteins were extracted from the leaves and analysed using electrophoresis to establish genetic relationships among species. Seven *Daucus* species have been identified to grow in Syria. These are *D. aureus*, *D. bicolor*, *D. carota*, *D. durieua*, *D. guttatus*, *D. littoralis*, and *D. muricatus*. Isozyme and total protein analysis, cluster analysis, and correlation matrix have revealed considerable genetic variation among studied *Daucus* species. Wild carrot (*D. carota*) came in one group with its cultivated form (*D. carota* ssp. *sativus*) and the closest species to them was *D. guttatus*. Species *D. bicolor* and *D. durieua* were in the same group and *D. aureus* and *D. littoralis* were in another group farther from the previous groups. The farthest species on the genetic tree was *D. muricatus*.

Key words: *Daucus*; carrot; isozyme; protein; Syria

Introduction

Daucus carota L. is an erect, tap-rooted herb, that belongs to the Apiaceae family and Apiales order. Although it can occur as an annual or short-lived perennial, the species is typically a biennial that bears a rosette of leaves in the first season (Dale 1974).

It is believed that carrots originated in Afghanistan and possibly northern Iran and Pakistan (Banga 1957). Orange carrots prevail worldwide despite the presence of some white types of carrots in Western and Eastern Europe (used as animal feed). Red carrots are found in Japan, yellow in the Middle East, and some purple, yellow and red carrots are found from Turkey to India and China (Simon 1999).

Carrots are consumed worldwide. World production was estimated at 24 million metric tons in the year 2005 (FAO, 2005). China comes first in carrot production (5 million) followed by USA and Russia (1 million each). Carrots can tolerate low temperature, salinity, weeds, poor soil, and low pH (Duke 1983).

Conventional classification of the genus *Daucus* relied on morphological characteristics of the stem, leaf, bract, inflorescence, flower, and fruit including primary ridges, vallecular spines, and secretory vesicle anatomy (as referenced by Vivek & Simon 1998, 1999).

Sáenz Laín (1981) classified 20 *Daucus* species into five sections: *Daucus* L. (12 species), *Anisactis* DC. (3 species), *Platyspermum* DC. (3 species), *Chrysodaucus* Thell. (one species), and *Meiodes* Lange (one species).

Although carrot (*Daucus carota* L.) comes from South Central Asia, most of other species in the genus *Daucus* originated around the Mediterranean Sea (Peterson & Simon 1986).

Syria is an important region for different plant genetic resources (wild and cultivated species) of the world, because of the diverse ecosystems and climatic conditions. Syria is considered as a centre of origin and biodiversity for many crops, feeds and fruit trees (Zohary 1962, 1973). It is one of the few core centres where numerous species of temperate-zone agriculture originated thousands of years ago, and where their wild relatives and landraces of enormous genetic diversity are still present. Estimates indicate that Syrian flora includes about 3150 species arranged in 919 genus in 133 families (Barkoudah et al. 2000). With regard to the genus *Daucus*, Syria is considered a centre of diversity (Sáenz Laín 1981; Mouterde 1986).

Biodiversity studies of plant species in Syria are very scarce and concentrate mainly on major crops or trees (MirAli & Nabulsi 2003, 2004; MirAli et al 2007), with the exception of a few studies on non crop plants (Aziz & Hamid 2006). Prior to the current study, no genetic, taxonomic, or ecological studies have been conducted in Syria on *Daucus* species or any other species in the Apiaceae family.

The objectives of this study were to investigate the distribution and to characterize *Daucus* species in Syria.

Table 1. Distribution of *Daucus* species in various regions of Syria with altitude and annual rainfall.

Code	Area	Altitude (m)	Rainfall (mm)	Daucus Species						
				carota	aureus	guttatus	littoralis	muricatus	Durieuia	bicolor
1	Yarmouk valley (south)	0–100	500	+	+	+	+		+	+
2	Sleem (south))	1039	350	+		+				
3	Sweida Aljabal (south)	1800	450	+++	+	+				
4	Zabadani (south west)	1200	500	+		+			+	
5	Surghaya (south west)	1360	600	+		+			+	
6	Tal Kalakh (central west)	275	900	+	+	+			+	
7	Alghab (central))	160–180	600–800	+	+	+				
8	Musiaf (central)	700	1000	+	+	+				
9	Karto (north)	400	500	+	+	+				
10	Orm Aljoz (north)	600	500	+	+	+			+	+
11	Coastal line	10–100	700–1000	++	+	+	+++		+	++
12	Kassab (coastal mountains)	650	1000	+		++	++		+	++
13	Slunfa (coastal mountains)	1100	1000	+		+		+	+	+

+ Low density, ++ Medium density, +++ High density

Material and methods

Sites of collection

Plants of the family Apiaceae, suspected to belong to the genus *Daucus*, were collected during the years 2000, 2001, and 2002 from road and field sides in regions of Syria that covered central, northern, southern, west, and coastal regions. The main sites of collection are briefly described in Table 1.

Collection and sampling

Fifty plants were collected from every suspected *Daucus* species from all aforementioned sites during April and May. Seeds were collected during August and September. The plants were brought to the laboratory and labelled with a number, site name, and date of collection. They were later sorted into groups determined on the basis of leaf shape, type of branching, number of branches, umbel shape, and umbel number. Off type plants were discarded.

Identification of plants

The seeds were sown in pots filled with peatmoss during March of 2001 and 2002 in a glasshouse at 8 °C under natural light. After emergence, temperature was gradually raised to 25 °C and maintained from flowering until fruit formation.

Plants growing in the glasshouse, and those collected from their natural habitats were used for identification. Plant groups from all sites were compared with each other. Five fruits were taken from the main umbel from every plant for comparison. Identification of plants was based on comparison of the fruit external and cross-section shapes in addition to spine shape and distribution on the fruit. Final species identification was determined using references (Sáenz Laín 1981; Mouterde 1986).

Isozyme and total protein analysis

Young leaves were collected from around 40 plants grown in the glasshouse at the average of 5 plants per identified *Daucus* species. Protein was extracted by grinding 1g of leaves in 1mL of extraction buffer (0.05 M Tris pH 6.8, 0.2% dithiothreitol (DTT), 1% polyvinylpyrrolidone-40 (PVP-40), 10% dimethylsulfoxide (DMSO), and 0.05% β -mercaptoethanol). Samples were centrifuged at 15 000 rpm for 15 minutes under 4 °C temperature. Five μ L glycerol/dye (0.5% bromophenol blue in 50% glycerol) was added to 100 μ L of supernatant. Sample proteins were separated using

0.75 mm slab polyacrylamide running gel (pH 8.8) and 4.5% stacking gel (pH 6.8). Samples were stacked (25 μ L sample/lane) at 25 mA and run for about 3 hours at 50 mA. Gels were stained for esterase (100 mg diazo blue and 50 mg α -naphthyl acetate dissolved in 20 mL of 95% ethanol, then added to 100 mL of 0.08 M Tris at pH 7.0) and acid phosphatase (100 mg diazo blue and 100 mg naphthyl phosphate dissolved in 100 mL 0.2 NaOAc at pH 5.0). For total protein, Coomassie stain was used (2.5 g brilliant Blue R dissolved in 90 mL glacial acetic acid, 450 mL 100% methanol, 450 mL distilled H₂O, destain: 180 mL glacial acetic acid, 600 mL 100% methanol, 1220 mL distilled H₂O) (Pooler & Simon 1994). All the steps were repeated 3 times to assure results.

Total protein bands, esterase (8 identified loci), and acid phosphatase bands (3 loci) were scored as present (1) or absent (0) and used to estimate the similarity among cultivars. Correlation matrix was calculated and a dendrogram, based on similarity coefficients, was generated with statistical program Statistica (Statsoft, 2001) using the unweighted pair group of arithmetic means procedure.

Results

Plant morphology and geographical distribution

Seven *Daucus* species have been determined to grow in the studied regions of Syria. Figure 1 shows a map of Syria illustrating areas where *Daucus* plants (of all species) have been found. A summary of the areas with their altitude, average annual rainfall, and relative distribution of *Daucus* species is presented in Table 1.

1 – *Daucus carota* L. Plants of this species are widely distributed in most of the investigated areas and at altitudes ranging from sea level to 1800 m.

The plants vary considerably in size and shape. The height of the plants ranges from 40 to 150 cm and exceeds 200 cm in some areas (Sweida & Jilleen). The stem is covered with coarse stiff hairs. The leaves are ovate, typically glabrous, although in some cases covered with soft hairs. The lower leaves are large and turn smaller at the top where they become bracts. The umbels, which consist of small white flowers, vary in size from 4 to 12 cm in diameter, with the upper umbels

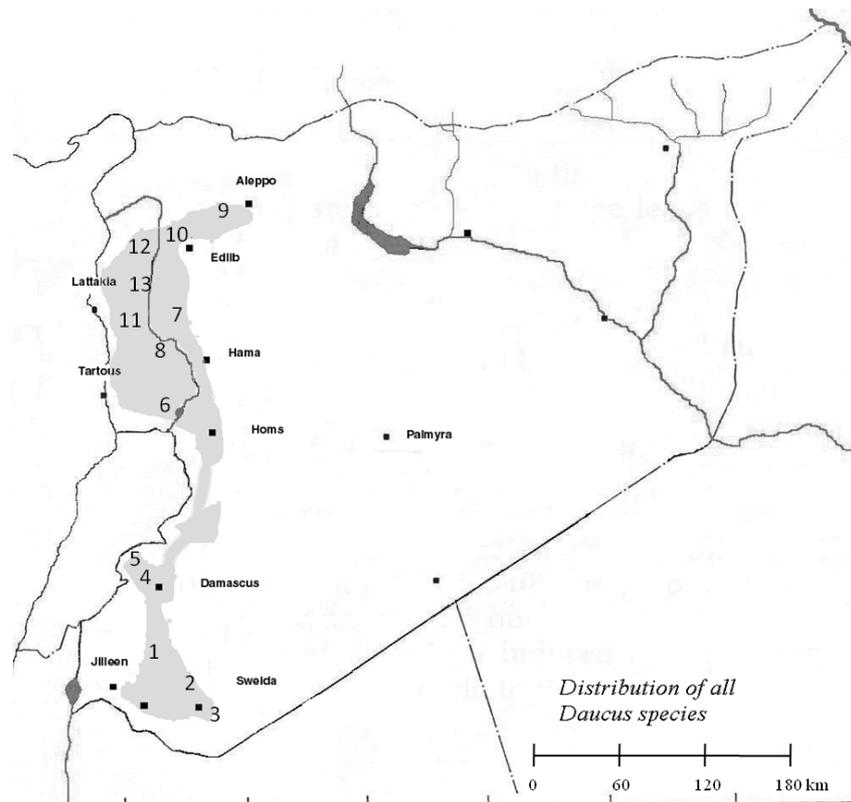


Fig. 1. Distribution of *Daucus* species in Syria.

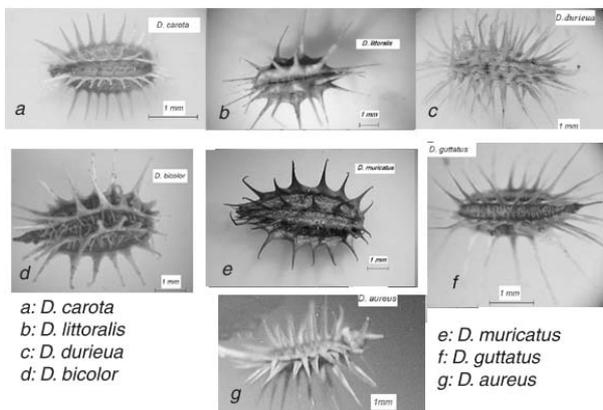


Fig. 2. Photos of fruits of various *Daucus* species found in Syria with their relative size.

being larger than the lower ones. They are surrounded by a circle of finely divided bracts, and become concave when the fruits mature. The flowers are white and change to purple or pinkish at the centre.

The fruits are small (3 × 2 mm), borne on relatively long (1 cm) pedicels. The primary ridges are conspicuous with two rows of simple hairs. The secondary ridges consist of rows of 10–12 spines (Fig. 2).

2 – *Daucus bicolor* SM (*Daucus broteri* Ten.): Plants of this species were found only in the coastal mountainous areas such as Slunfa, Salma, and Rabiaa, and near Tartous. Some plants were also found in Jilloen (southwest of the country close to Yarmouk valley).

The plants are erect or procumbent, branched, and may reach 40 cm. The stems are thin hispid and the leaves are oblong, alternate, and pinnatisect. Umbels are terminal, pedunculate 3–5 cm in diameter. Bracts are leaf-like, longer than the umbel but curve in at fruit maturity to become shorter. The fruits are elongated, ovoid (4–5 × 2–3 mm). The primary ridges are conspicuous with multi-seriate hairs. The secondary ridges consist of 7–8 rows of yellowish to light brown spines, longer than the width of the fruit (Fig. 2).

3 – *Daucus durieua* Lange (*Daucus subsessilis* Boiss): The species is found in slight density in the coastal mountainous areas and even with lesser density in the south and southwestern areas of the country.

The plants exist in two types. The first type, found in the coastal mountains, has many stems emerging from the base of the plant and reaches a maximum of 30 cm in height. The other type, found in south and southwest regions, has one simple branched stem and can reach 50 cm in height. The stems are covered with soft hairs. The leaves are alternate, pinnately divided into 3–5 segments and lanceolate with acute tips. The umbels are, in general, axillary, and terminal in the branched type. They vary in size, and are relatively smaller than umbels of other species. Bracts look like leaves, they are longer than the umbel and the petals are very small, white to yellowish. Fruits are ovoid with 4 × 2 mm dimensions. The primary ridges are inconspicuous, with a number of simple rows of hairs. The secondary ridges consist of rows of yellow spines longer than those of the fruit (Fig. 2).

Table 2. Spearman Rank Order Correlations for *Daucus* species and cultivated carrot

	<i>guttatus</i>	<i>carota</i>	<i>bicolor</i>	<i>littoralis</i>	<i>muricatus</i>	<i>durieua</i>	<i>cultivated</i>	<i>aureus</i>
<i>guttatus</i>	1.00	0.62*	0.09	0.22	-0.21	0.37	0.53*	-0.09
<i>carota</i>	0.62*	1.00	0.19	0.34	-0.05	0.51*	0.89*	0.26
<i>bicolor</i>	0.09	0.19	1.00	-0.26	-0.15	0.64*	0.07	0.06
<i>littoralis</i>	0.22	0.34	-0.26	1.00	-0.21	0.11	0.45	0.68*
<i>muricatus</i>	-0.21	-0.05	-0.15	-0.21	1.00	-0.28	-0.13	-0.07
<i>durieua</i>	0.37	0.51*	0.64*	0.11	-0.28	1.00	0.42	0.04
<i>cultivated</i>	0.53*	0.89*	0.07	0.45	-0.13	0.42	1.00	0.37
<i>aureus</i>	-0.09	0.26	0.06	0.68*	-0.07	0.04	0.37	1.00

* Significantly correlated at $P < 0.05$

4 – *Daucus guttatus* SM. This species is widely distributed in all studied areas along with *Daucus aureus*, however, a lower density is noticed in the area situated south west of Damascus and the southern part of the country.

The plants are generally short, reaching a maximum of 30 cm in natural habitat but may reach 100 cm when grown in a glasshouse. The plants are erect and branched (usually from the base). The stems are thin, hispid and the leaves pinnate divided to 3 segments and further are divided into smaller ones. Umbels are terminal, pedunculate, very small and the bracts divided and longer than the umbel. The petals are very small, white to yellowish and the fruits are small $3-4 \times 1-2$ mm. The primary ridges are conspicuous, with number of simple rows of hairs. The secondary ridges consist of rows of about 8 white spines longer than the width of the fruit (Fig. 2).

5 – *Daucus aureus* Desf. Plants of this species were found growing in areas spreading from Aleppo in the north to Jilleen in the south, especially around Dara'a. The species was also present in lesser density in Zabadani westward of Damascus and Sweida region. No plants of this species were found in the coastal region. Average height of the plants exceeded 50 cm in some areas but in others it reached only 15 cm. The stem is erect, branched from the base or above into, usually, two branches. The leaves are large, oblong, pinnate and alternate. The umbels are terminal, pedunculate and large (about 7 cm). The bracts are divided and shorter than the umbel. The fruits are elongated, ovoid $5-7 \times 2$ mm, with cone-shaped bulge at the base. The primary ridges are inconspicuous. The secondary ridges consist of rows of 7-8 yellow spines as long as the width of the fruit, but become shorter in fruits located in the centre of the umbel (Fig. 2).

6 – *Daucus littoralis* Sm. This species is distributed only in the coastal mountainous areas with the exception of sporadic existence of plants in Jilleen in the south of the country.

The plants can reach 50 cm in natural habitat and as much as 110 cm in the glasshouse. They are erect branched or procumbent, with hispid or rarely glabrous stems. The leaves are pinnate, lanceolate with acute tips. The umbels can be terminal or axillary, large (7 cm), with long peduncles and concave as fruits mature.

Bracts are simple and very short or divided and long. The petals are white to yellowish and the fruits are oval and large ($5-6 \times 4$ mm), dilated at the base. The primary ridges are conspicuous, with rows of thick hairs. The secondary ridges consist of rows of about 7-8 yellow spines, shorter than the width of the fruit and end with a coned bulge (Fig. 2).

7 – *Daucus muricatus* (L). Previous studies did not report the existence of this species in Syria (Sáenz Laín 1981; Mouterde 1986). However, we found plants of this species to grow in the coastal mountainous areas (Slunfa, Salma, Doreen, and Kassab) and in the north-west area near the Turkish border. These plants were seen only on gravelly soils or between rocks.

The plants are generally short (reach only 30 cm), erect branched and thin hispid. The leaves are alternate, pinnatisect, divided into oval segments. The umbels are terminal or axillary, large (6-8 cm), with relatively long peduncles. The bracts are divided and much shorter than the umbel. The petals are yellowish and large. The fruits are ovoid and large (7×3 mm). The primary ridges are thick, with 2 rows of silvery rigid hairs, longer than the fruit width. The secondary ridges have a row of spines twice longer than the fruit width (Fig. 2).

Genetic relationships among species

Isozyme and total protein electrophoretic analysis, cluster analysis, and correlation matrix have revealed considerable genetic variation among studied *Daucus* species. Correlation matrix (Table 2) for the seven studied *Daucus* species and cultivated carrot revealed that *Daucus carota* is significantly correlated ($P < 0.05$) with cultivated carrot, *Daucus guttatus*, and *Daucus durieua* ($r = 0.89, 0.62$, and 0.51 respectively), while *Daucus muricatus* was not significantly correlated with any of the other species. Also, it is clear from the cluster tree (Fig. 3) that *Daucus* species fell into several groups. *D. carota* and its cultivated form (*D. carota* ssp. *sativus*) were in the same group with *D. guttatus* being close to them in a larger group. Species *D. bicolor* and *D. durieua* fell in one group and the other two species *D. littoralis* and *D. aureus* in another group farther from the previous groups. The farthest species on the genetic tree was *D. muricatus*.

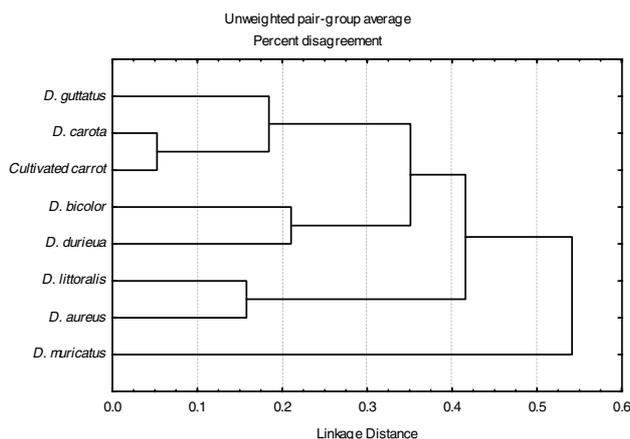


Fig. 3. Tree diagram for 7 *Daucus* species and cultivated carrot.

Discussion

Daucus species have not, generally, received much attention in plant biodiversity studies conducted in the region. Only a few studies have been published that dealt with *Daucus* species in Syria (Sáenz Lain 1981; Mouterde 1986). Mouterde (1986), studied the flora of Lebanon and Syria, and reported the existence of only six *Daucus* species (*carota*, *durieua*, *aureus*, *guttatus*, *bicolor*, and *littoralis*) in Syria. Also, the study of Sáenz Lain (1981) did not mention the existence of *D. muricatus* in Syria but only in Turkey.

Our study, on the other hand, confirmed the existence of seven *Daucus* species, *D. aureus*, *D. bicolor*, *D. carota*, *D. durieua*, *D. guttatus*, *D. littoralis*, and *D. muricatus*, in Syria. Moreover, we found the aforementioned species to grow in wider areas of the country than mentioned by previous studies.

Daucus species were found to grow, in Syria, in wide range of geographical areas and different climates ranging from sea level to 1800 m. However, wider distribution was recorded in areas receiving over 500 mm of annual rain (Table 1).

The richest area in *Daucus* species was the coastal line region where all species, except *D. muricatus*, were found. This area is plain, fertile and rich in terms of agriculture. It stretches about 180 km long. Plant cover consists of shrubs, annual and perennial weeds such as *Ephedra*, *Inula*, *Lolium*, *Trifolium* as well as *Daucus* species.

D. carota and *D. guttatus*, were the only *Daucus* species found in all surveyed areas. Plants of *D. carota* varied considerably in size among studied areas. The largest plants (about 200 cm in height) and the highest density of plants were found in Sweida, Aljabal, a mountainous area located in the south of the country. The area is characterized with cool summers, very cold winters and average humidity. Plant cover in this area is dominated by *Aegilops*, *Carthamus*, *Centaurea*, *Trifolium*, *Triticum* species, in addition to *Daucus*.

The poorest area in the distribution of *Daucus* species was Sleem where only 2 species, *D. carota* and

D. guttatus, were found. This area is located in the south of the country with an altitude of about 1 000 m. The weather is characterized with low humidity, hot summers and cold winters. Average annual rainfall is only 350 mm. The terrain is rough with little vegetation, consisting mainly of species such as *Caucalis*, *Picnomon*, *Poa*, *Scariola*, *Stipa* species and *Daucus*.

Despite its existence in all surveyed areas, *D. guttatus* was largely found in Kassab, a mountainous area located in the coastal area with an altitude reaching 650 m and an average rainfall of about 1 000 mm per year. The terrain is rocky, covered with good vegetation consisting of annual and perennial species as well as shrubs and forest trees such as *Bellardia*, *Bromus*, *Cotinus*, *Eleagnus*, *Kalmia*, *Koeleria* species as well as *Daucus*.

The least disseminated species of all recorded *Daucus* species in Syria was *D. muricatus*. It was found only in the area of Slunfa, another coastal mountain area, higher than Kassab (1 100 m), with an annual 1 000 mm of rain. The terrain is very rough, covered with annual and perennial species as well as shrubs and forest trees such as *Abies*, *Cedrus*, *Cistus*, *Juniperus*, *Ruscus*, species and *Daucus*.

In addition to the morphology and geographical-distribution study of *Daucus* species in Syria, a genetic study was conducted to find out genetic relationships among those species. The isozyme and protein electrophoretic analysis has revealed a close relationship between *D. littoralis* and *D. aureus* and a distant relationship between *D. muricatus* and the other *Daucus* species growing in Syria. This confirms the findings of other researchers (Vivek & Simon 1998; 1999) who placed *D. muricatus* in a group different from the group of *D. broteri* (*bicolor*), *D. aureus*, and *D. littoralis* species.

Our study has enabled us to learn more about the environmental conditions (temperature, rainfall, altitude) typical to the growth of *Daucus* species in Syria and their relative distribution, which should be useful in their preservation and making use of them.

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