



The effect of water deficiency and salinity on the growth and quality of fresh dill (*Anethum graveolens* L.) during autumn and spring cultivation

D. Tsamaidi^a, D. Daferera^b, I.C. Karapanos^a, H.C. Passam^{a,*}

^aAgricultural University of Athens, Laboratory of Vegetable Production, Iera Odos 75, 11855 Athens, Greece.

^bAgricultural University of Athens, Laboratory of Vegetable Production, Iera Odos 75, 11855 Athens, Greece.

*Corresponding author. E-mail: passam@aua.gr

Received 1 April 2016; Accepted after revision 8 September 2016; Published online 17 January 2017

Abstract

The aim of this experiment was to determine the response of dill (*Anethum graveolens* L.) to water deficiency and salinity. In spring, dill biomass decreased with increasing water deficiency due to a reduction in the number and mean weight of leaves per plant. The concentrations of chlorophyll, carotenoids, vitamin C and total phenolics within the leaves were unaffected by water stress, but the Cl, Na and K content decreased. Salinity had no effect on plant growth in the spring, except at the highest level (8 dS/m⁻¹), where a decrease in biomass occurred. The chlorophyll and total phenolics content of leaves rose in the autumn with increasing salinity, whereas vitamin C and carotenoids were unaffected. In spring, increasing salinity caused fluctuations in the chlorophyll and vitamin C content of the leaves and a decrease in total phenolics. The concentrations of chlorophyll and antioxidants were higher in spring than in autumn at all levels of salinity. The essential oil content was also higher in the spring than in the autumn, irrespective of salinity. A relative decrease in dill ether within the herb oil under increasing salinity was compensated for by an increase in α -phellandrene. In the flower oil, increasing salinity caused a decrease in the relative concentrations of both α -phellandrene and dill ether, which was compensated for by an increase in carvone. Overall, dill appears more resistant to salinity than to water stress, but the season of cultivation has the most important effect on both yield and quality.

Keywords: Biomass; Chlorophyll; Carotenoids; Vitamin C; Phenolics.

Introduction

Water availability is a major environmental factor for the productivity of crop plants and water shortage has been estimated to adversely affect the agricultural usage of 25% of the world's land (Delfine et al., 2005). Water deficiency not only limits plant growth and survival but also induces various physiological and metabolic responses, such as stomatal closure, a decline in growth rate, solute and antioxidant accumulation, a reduction in photosynthesis and transpiration and the expression of stress specific genes (Jones and Tardien, 1998; Hughes et al., 1989). The stress response depends on the severity of drought or water shortage (Sarker et al., 2005) and varies with the species, cultivar and plant density (Fatima et al., 2000).

In the case of aromatic plants, water deficit decreased the plant height, fresh and dry weight of *Satureja hortensis* (Baher et al., 2002) and reduced the leaf area of dill (Charles et al., 1995), as well as affecting the oil yield and composition of sweet basil (Simon et al., 1992), rosemary (Singh and Ramesh, 2000) and anise (Zehtab-Salmasi et al., 2001). Similarly in geranium, water stress had a negative impact on biomass and essential oil yield as the intervals between irrigations increased (Putievsky et al., 1990).

Salinity in the soil and/or irrigation water is also a serious environmental problem that can cause drastic changes in the growth, metabolism and productivity of crops (Jaleel et al., 2008; Zhu, 2001). It is estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity and sodium content (Mostafazadeh-Fard et al., 2007). The growth and yield of many crop species are sensitive to salinity, the deleterious effects of which are attributed to increased osmotic pressure within the root zone and ion toxicity. This leads to nutritional imbalance, a reduction in photosynthetic efficiency, stomatal closure, ion homeostasis, osmolyte accumulation and an increase in the generation of reactive oxygen species (Munns, 2002; Parida and Das, 2005; Parvaiz and Satyawati, 2008). Salt stress may also induce changes in plant morphology (Amirjani, 2010).

All plants tolerate salinity up to a certain threshold level without any yield reduction, the degree of tolerance relating to the developmental stage of the plant at the time of exposure to stress (Adam, 1990). Thereafter, a further increase in salinity significantly reduces yield (Ozturk et al., 2004). Dill (*Anethum graveolens* L.), an annual aromatic herb of the *Apiaceae* grown widely throughout Europe, America and Asia for use as a fresh herb and for the production of essential oil, is reported to be relatively tolerant to salinity (Ghassemi-Golezani et al., 2011; Udagawa, 1995; Mehr et al., 2012; Nourani Azad et al., 2008; Gururaja Rao et al., 2001a,b). However, plant growth is restricted by water stress (Mehta et al., 2012; Ghassemi-Golezani et al., 2008). Because there is little or no available information concerning the effects of salinity and water stress on dill growth in relation to the season of cultivation or on the quality of the fresh herb, the experiments described in the present paper were undertaken.

Materials and Methods

Cultivation and treatments

The study was carried out at the Agricultural University of Athens (37° 58' 55.83'' N, 23° 42' 16.69'' E). Seeds of dill (*Anethum graveolens* L. cv. Ducat) were sown in trays containing a commercial peat compost (Klasmann TS-2, Klasmann-Deilmann GmbH, Geeste, Germany) on 02 October 2011 (autumn sowing of salinity experiment of year 1), on 08 January 2012 (spring sowings of the salinity and water stress experiments of year 1) and on 03 February 2013 (spring sowing of the water stress experiment). Stress by water deficiency was not applied during the autumn since the climatic conditions of southern Greece (relatively high rainfall in comparison with the spring) rarely cause water deficiency during this period.

At the stage of 3-4 true leaves, the plants were transplanted to 11 L pots containing a (1:1v/v) mixture of peat and perlite (Perloflor P4, Perlite Hellas, Piraeus, Greece) enriched with 150 g potassium monophosphate, 40 g potassium sulphate, 20 g magnesium sulphate, trace elements and 300 g marble dust per m³. The pots contained four plants each and were spaced outdoors at 50 × 50 cm. During cultivation, the plants were fertilized with 300 ppm N-P-K fertilizer (20-20-20) within the irrigation water.

Water stress was applied to plants by delaying irrigation until the moisture level of the substrate at irrigation (saturation) decreased to the desired level according to the indications of soil tensiometers (Irrometer-Moisture Indicator, Irrometer, Riverside, California) placed in the pots. Three irrigation regimes were employed: (1) irrigation when the tensiometer readings reached 20 centibars (adequate water supply – control) and (2, 3) two levels of water deficiency, when irrigation was applied (2) at 30-40 centibars (WS40) and (3) at 50-60 centibars (WS60) on a scale where 0 centibars = soil saturation and 100 centibars = dry soil. In each treatment, 60 plants were used.

To study the effect of salinity, NaCl was applied to plants in the form of aqueous solutions. The electrical conductivity (EC) levels applied were 0.63 (control), 2, 4, 6 and 8 dS m⁻¹. In each treatment, 120 plants were used. The EC of the control was that of the irrigation water and added fertilizer, while in the other treatments a stock solution of NaCl (1M) was added to the irrigation water in quantities sufficient to achieve the desired EC. Salinity was applied once the plants had formed the 4th to 6th true leaf. Initially, irrigation was applied at a rate of 0.5L per pot once a week, but with increasing growth and temperature (in the spring) the quantity was progressively increased to 1L every 2-3 days. To avoid run-off, plastic dishes were placed beneath each pot.

The plants of all treatments were harvested in the early morning: 89 and 106 days after sowing the autumn and spring crops of the salinity experiment and 93 days after sowing the crop for the water stress experiment, just prior to flowering. Additionally, in the autumn crops, a few plants were permitted to flower and the flowers and fruits/seeds collected separately for oil analysis. The plants were cut 1cm above the substrate and, following the removal of any old, senesced leaves, immediately transferred to the laboratory.

Laboratory analysis

On arrival in the laboratory, the fresh weight of foliage and the number of leaves per plant were recorded. For dry matter determination five samples of leaves from each treatment were randomly selected at harvest. The leaves were dried to constant weight at 72 °C and ground in a MF 10 Basic mill (IKA-Werke, GmbH & Co. KG, Staufen, Germany) to mesh size < 0.25 mm. Quality characteristics (chlorophylls, carotenoids, vitamin C and total phenolics) in the leaves were determined colorimetrically using a Perkin Elmer Model Lambda 1A spectrophotometer (Perkin Elmer, Waltham, MA, USA.). In each treatment five replicates were used. Chlorophyll a, chlorophyll b and total chlorophyll concentrations of the leaves were measured using the method of Arnon (1949), carotenoids were determined according to Kirk and Allen (1965), vitamin C (L-ascorbic acid) was measured following the method of Bajaj and Kaur (1981), while total phenolics were measured by the Folin Ciocalteu method according to Lisiewska et al. (2006).

The inorganic ion content (Cl, Na and K) of leaves was determined in year 2. Cl was determined by titration according to Eaton et al. (1995), while Na and K were determined using flame emission spectroscopy (Sherwood Model 410, Cambridge, UK), according to Horneck and Hanson (1998).

Because of a lack of plant material in the water stress experiment, essential oil extraction was performed only from the plants subjected to salinity.

Essential oil extraction and analysis

Immediately after harvest, leaves (or additionally some flowers and fruits/seeds in the autumn crops) were placed in sealed, airtight plastic food-bags and stored at -20°C until essential oil extraction. Essential oil yield was measured with the use of hydro-distillation, using a Clevenger apparatus filled with 100 g of plant material for 3 hours (Petropoulos et al., 2008). Essential oils were analyzed by gas chromatography (GC) using a Hewlett Packard 5890 II gas chromatograph equipped with a FID detector and a HP-5MS capillary column ($30\text{ m} \times 0.25\text{ mm}$, film thickness $0.25\text{ }\mu\text{m}$). Quantitative data were obtained electronically from FID area percent data without the use of correction factors. Gas Chromatography/Mass Spectrometry (GC/MS) analysis was performed under the same conditions as GC using a Hewlett Packard 5890 II GC equipped with a Hewlett Packard 5972 mass selective detector in the electron impact mode (70 eV). Tentative identification of the compounds was based on a comparison of their relative retention time and mass spectra with those of pure standards and the NBS75K library data of the GC/MS system and literature data (Adams, 2001). A minimum of three assays were performed for each treatment.

Statistical methods

The results were subjected to analysis of variance (ANOVA) and means (of 5 replications per experimental treatment) were compared by the application of Duncan's multiple range tests using the statistical package SPSS Statistics 22.

Results

Influence of water deficiency

Plant height decreased with increasing water deficiency in year 1, but not in year 2 where, due to adverse climatic conditions (temperatures in excess of 30°C during April and May), plant growth was less than in year 1 irrespective of the level of water deficiency. The number of leaves plant^{-1} , plant biomass (fresh weight) and leaf weight plant^{-1} all decreased with increasing water deficiency in both years (Table 1).

Table 1. The effect of water stress on plant height and weight, leaf number and weight per plant.

Season	Irrigation level	Plant height (cm)	Number of leaves plant^{-1}	Plant weight (g plant^{-1})	Leaf weight (g plant^{-1})				
Spring 2012	Control	45.9	a	6.2	a	42.4	a	30.6	a
	WS40	37.0	b	5.0	b	26.2	b	20.4	b
	WS60	40.0	b	4.6	b	21.8	c	15.8	c
Spring 2013	Control	25.2	a	5.1	a	12.3	a	9.2	a
	WS40	24.9	a	4.3	b	9.1	b	5.7	b
	WS60	24.6	a	3.5	c	5.8	c	2.4	c

Means for each season in columns followed by the same letter are not significantly different at $P=0.05$.

The concentration of chlorophyll (a, b and total) was unaffected by water deficiency, with the exception of chlorophyll a in year 1, which tended to increase under moderate stress (WS 40) (Table 2). Similarly, there were no significant changes in the concentrations of carotenoids, vitamin C or total phenolics in relation to water deficiency in either year (Table 3). The concentrations of chlorine and sodium in the leaves decreased with increasing water deficiency, as did the concentration of potassium (at WS 60) (Table 4).

Table 2. The effect of water stress on the chlorophyll (a, b and total) concentrations in the leaves.

Season	Irrigation level	Chlorophyll a		Chlorophyll b		Chlorophyll total	
		(mg 100 g f.w. ⁻¹)					
Spring 2012	Control	232.3	b	106.1	a	338.3	b
	WS40	298.6	a	151.3	a	449.9	a
	WS60	235.2	b	137.2	a	372.5	ab
Spring 2013	Control	200.7	a	100.9	b	301.7	a
	WS40	199.5	a	109.5	ab	309.1	a
	WS60	192.3	a	119.4	a	311.7	a

Means for each season in columns followed by the same letter are not significantly different at P=0.05.

Table 3. The effect of water stress on the concentrations of carotenoids, vitamin C and total phenolics in the leaves.

Season	Irrigation level	Carotenoids		Vitamin C		Total phenolics	
		(mg 100 g f.w. ⁻¹)					
Spring 2012	Control	28.6	a	324.4	a	239.3	a
	WS40	27.5	a	333.8	a	233.8	a
	WS60	36.2	a	370.4	a	249.9	a
Spring 2013	Control	27.9	a	318.8	a	278.3	a
	WS40	24.2	a	311.7	a	247.3	a
	WS60	23.4	a	305.8	a	252.7	a

Means for each season in columns followed by the same letter are not significantly different at P=0.05.

Table 4. The effect of water stress on the concentrations of Cl, Na and K in the leaves.

Season	Irrigation level	Cl ⁻		Na ⁺		K ⁺	
		(mg 100 g f.w. ⁻¹)					
Spring 2013	Control	18.8	a	39.7	a	549.3	a
	WS40	9.3	b	16.2	b	589.3	a
	WS60	9.7	b	13.6	b	329.8	b

Means within each column followed by the same letter are not significantly different at P=0.05.

Influence of salinity

The effect of salinity on plant growth differed between the seasons of cultivation. Thus, in the autumn plant height increased under the presence of salinity at 4-8 dS m⁻¹. Similarly, there was an increase in the number of leaves per plant (although not always to a statistically significant level) and in plant biomass at all levels of salinity. By contrast, in the spring there was no effect of salinity on plant height or plant biomass (except for a reduction in biomass at 8 dS m⁻¹), while the number of leaves per plant decreased at the highest salinity levels (6-8 dS m⁻¹) (Table 5).

Table 5. The effect of salinity on plant height, leaf number and leaf weight per plant.

Season	Salinity level (dS m ⁻¹)	Plant height (cm)		Leaf number plant ⁻¹		Leaf weight (g plant ⁻¹)	
Spring	0.63	44.5	a	6.5	a	54.9	a
	2.0	40.5	a	6.1	ab	57.0	a
	4.0	40.3	a	6.2	a	58.2	a
	6.0	43.4	a	5.7	bc	59.1	a
	8.0	40.2	a	5.5	c	41.8	b
Autumn	0.63	48.0	b	6.7	b	38.5	b
	2.0	51.1	ab	7.7	a	52.4	a
	4.0	51.9	a	6.9	b	55.3	a
	6.0	53.6	a	7.6	a	61.5	a
	8.0	52.8	a	7.3	ab	55.7	a

Means for each season within each column followed by the same letter are not significantly different at P=0.05.

The concentration of total chlorophyll in the leaves increased significantly at the higher salinity levels (4-8 dS m⁻¹) in the autumn, whereas in the spring an increase in leaf chlorophyll concentration was observed at low levels of salinity (2-4 dS m⁻¹), but at higher levels (6-8 dS m⁻¹) decreased, though not to a significant level at 8 dS m⁻¹ (Table 6). The leaf chlorophyll concentration in the spring was higher than in the autumn at all levels of salinity except 6 dS m⁻¹, where the difference was not statistically significant. In consequence, the leaves of plants grown in spring were darker green in colour than those grown in the autumn irrespective of the level of salinity.

Table 6. The effect of salinity on the total chlorophyll concentration in the leaves.

Salinity level (dS m ⁻¹)	Total chlorophyll (mg 100 g f.w. ⁻¹)			
	Spring		Autumn	
0.63	330.5	c*	155.1	c
2.0	542.1	a*	144.3	c
4.0	408.2	b*	191.3	ab
6.0	184.5	d	164.0	bc
8.0	287.7	c*	204.3	a

Means for each season within each column followed by the same letter are not significantly different at P=0.05. Means followed by an asterisk (*) differ significantly (P=0.05) between the two crops (autumn and spring).

The concentration of carotenoids in the leaves of plants from the spring cultivation increased at the level of 2-4 dS m⁻¹ but was not affected at 6-8 dS m⁻¹, while in autumn there was an increase only at 6 dS m⁻¹ (Table 7). In contrast, the vitamin C content decreased under conditions of salinity in both seasons, although not always to a statistically significant degree. In contrast, whereas in the spring the concentration of total phenolics decreased at all levels of salinity, in the autumn an increase was observed at the highest salinity levels (6-8 dS m⁻¹) (Table 7). When comparing the results from the two seasons of cultivation, it is apparent that at each level of salinity the concentrations of carotenoids, vitamin C and total phenolics within the leaves was almost invariably higher in the spring than that at the corresponding levels of salinity in the autumn.

Table 7. The effect of salinity on the concentrations of carotenoids, vitamin C and total phenolics in the leaves.

	Salinity level	Carotenoids		Vitamin C		Total phenolics	
	(dS m ⁻¹)			(mg 100 g f.w. ⁻¹)			
Spring	0.63	24.9	b*	344.9	a*	301.0	a*
	2.0	39.9	a*	260.9	b*	265.2	b*
	4.0	34.0	a*	325.7	ab*	196.1	c
	6.0	20.2	b	114.4	c	248.2	b*
	8.0	26.1	b*	255.0	b*	264.3	b*
Autumn	0.63	17.0	b	146.7	ab	229.7	b
	2.0	14.8	b	166.7	a	199.5	b
	4.0	17.4	b	121.6	b	181.3	c
	6.0	23.2	a	114.9	b	303.5	a
	8.0	17.9	b	139.6	ab	325.3	a

Means for each season within each column followed by the same letter are not significantly different at P=0.05. Means followed by an asterisk (*) differ significantly (P=0.05) between the two crops (autumn and spring).

The inclusion of increasing amounts of NaCl in the irrigation water caused a progressive increase in the concentrations of sodium and chlorine within the leaves during both seasons of cultivation. In contrast, the concentration of potassium fluctuated with increasing salinity in the spring but progressively decreased in the autumn (Table 8). At corresponding levels of salinity, the concentration of all three ions was higher in leaves in the spring than in the autumn (except Na at 8 dS m⁻¹).

Table 8. The effect of salinity on the concentrations of Cl, Na and K in the leaves.

	Salinity level	Cl ⁻	Na ⁺		K ⁺		
	(dS m ⁻¹)		(mg 100 g f.w. ⁻¹)				
Spring	0.63	29.2	d	19.7	e*	1284.2	b*
	2.0	53.6	cd*	68.0	d*	968.0	d*
	4.0	74.6	bc*	174.6	c*	1099.4	c*
	6.0	94.9	b*	387.7	a*	1238.8	b*
	8.0	200.4	a*	278.3	b	1446.6	a*
Autumn	0.63	17.6	d	15.0	d	744.0	a
	2.0	26.7	d	31.1	d	661.6	b
	4.0	43.4	c	134.1	c	536.5	c
	6.0	64.5	b	233.5	b	460.0	d
	8.0	76.5	a	306.0	a	394.3	d

Means for each season within each column followed by the same letter are not significantly different at P=0.05. Means followed by an asterisk (*) differ significantly (P=0.05) between the two crops (autumn and spring).

The yield of essential oil from the leaves of dill (herb oil) in the autumn was 0.10-0.14 ml 100 g fresh weight⁻¹, irrespective of the level of salinity (Table 9). In spring, the oil yield was relatively higher, ranging between 0.13 and 0.37 ml 100 g fresh weight⁻¹ at the lower levels of salinity (<3 dS m⁻¹), but rising to 1.29 ml 100 g fresh weight⁻¹ at 4.5 dS m⁻¹, consistent with an increase in aroma of the spring-grown plants compared with those in the autumn (Table 9). The principal constituents of dill herb oil in the autumn crop were α -phellandrene (63.27-70.16 mg 100 g fresh weight⁻¹), β -phellandrene (12.08-12.88 mg 100 g fresh weight⁻¹) and dill ether (8.80-12.17 mg 100 g fresh weight⁻¹) (Table 10). In contrast, the principal constituents of dill flower oil were α -phellandrene (15.85-35.26 mg 100 g fresh weight⁻¹), limonene (32.00-37.55 mg 100 g fresh weight⁻¹) and carvone (22.89-40.58 mg 100 g fresh weight⁻¹) (Table 11) while those of the seeds were limonene (39.37-42.67 mg 100 g fresh weight⁻¹) and carvone (40.34-54.98 mg 100 g fresh weight⁻¹) (Table 12).

The most significant effect of increasing salinity on dill herb oil composition was that it caused a progressive increase in the relative concentration of α -phellandrene, which is a major aroma constituent, but a small decrease in another aroma constituent: dill ether (Table 10). However, in the case of flower oil, increasing salinity caused a decrease in the relative concentrations of both α -phellandrene and dill ether, which was compensated for by an increase in carvone (Table 11), whereas in seed/fruit oil there was virtually no effect of salinity on the oil constituents (Table 12).

Table 9. The effect of salinity on dill oil yield (ml oil 100 g fresh weight⁻¹).

Salinity level (dS m ⁻¹)	Spring			
	Leaves (lamina)		Flowers	
0.63	0.37		0.73	
1.5	0.13		0.93	
3.0	0.20		1.09	
4.5	1.29		0.96	

	Autumn			
	Leaves (lamina)	Flowers	Fruits	Seeds
0.63	0.12 ^a (d)	0.74 ^b (c)	1.10 ^c (b)	2.22 ^b (a)
2.0	0.10 ^a (d)	0.95 ^a (c)	1.22 ^c (b)	3.56 ^a (a)
4.0	0.13 ^a (c)	0.84 ^a (b)	2.97 ^b (a)	nd*
6.0	0.14 ^a (c)	0.93 ^a (b)	4.44 ^a (a)	nd

Means for each season within each column followed by the same letter are not significantly different at P=0.05. Means for each salinity level followed by a different letter in parenthesis are significantly different (P=0.05) between the plant parts.

*nd: not determined

Table 10. The principal constituents of dill herb oil (mg 100 g fresh weight⁻¹) of the autumn crop in relation to salinity.

Oil composition	R.T. (min)	0.63 dS m ⁻¹	2.0 dS m ⁻¹	4.0 dS m ⁻¹	6.0 dS m ⁻¹
α -thujene	8.10	0.13 ^b	0.15 ^b	0.23 ^a	0.23 ^a
α -pinene	8.37	1.30 ^b	1.35 ^b	2.06 ^a	2.13 ^a
Sabinene	9.52	nd*	0.05 ^b	0.05 ^b	0.06 ^a
β -pinene	9.90	0.01 ^c	0.06 ^b	0.16 ^a	0.14 ^a
β -myrcene	10.40	0.54 ^b	0.64 ^b	0.81 ^a	0.81 ^a
α -phellandrene	11.50	63.27 ^b	66.95 ^{ab}	69.77 ^a	70.16 ^a
π -cymene	11.90	0.71 ^a	1.05 ^a	0.97 ^a	0.81 ^a
β -phellandrene	12.30	12.08 ^a	12.85 ^a	12.70 ^a	12.88 ^a
Terpinolene	15.90	0.23 ^a	0.13 ^a	0.16 ^a	0.13 ^a
Dill ether	19.50	12.17 ^a	11.06 ^{ab}	8.80 ^b	9.76 ^b
Germacrene D	34.53	0.25 ^a	0.47 ^a	0.44 ^a	0.45 ^a

Means within each row followed by the same letter are not significantly different at P=0.05.

nd: not determined

Table 11. The principal constituents of dill flower oil (mg 100 g fresh weight⁻¹) of the autumn crop in relation to salinity.

Oil composition	R.T. (min)	0.63 dS m ⁻¹	2.0 dS m ⁻¹	4.0 dS m ⁻¹	6.0 dS m ⁻¹
α -pinene	8.37	0.79 ^a	0.67 ^a	0.36 ^b	0.42 ^b
β -myrcene	10.40	0.37 ^a	0.36 ^a	0.29 ^a	0.32 ^a
α -phellandrene	11.50	35.26 ^a	32.41 ^a	17.66 ^b	15.85 ^b
Limonene	11.89	32.00 ^a	33.53 ^a	34.10 ^a	37.55 ^a
π -cymenene	14.20	0.08 ^a	0.07 ^a	0.07 ^a	0.06 ^a
Dill ether	19.50	10.17 ^a	5.80 ^b	3.90 ^c	4.26 ^{bc}
Cis-dihydro carvone	20.16	0.31 ^a	0.31 ^a	0.30 ^a	0.25 ^a
Trans-dihydro carvone	21.48	1.68 ^b	2.34 ^a	2.17 ^{ab}	1.68 ^b
Carvone	21.78	22.89 ^b	32.64 ^a	40.58 ^a	36.32 ^a
Trans-carvyl acetate	23.74	0.08 ^a	0.04 ^a	0.15 ^a	0.07 ^a
Cis-carvyl acetate	24.05	0.23 ^a	0.10 ^a	0.10 ^a	0.15 ^a
Germacrene D	34.53	0.10 ^a	0.05 ^a	0.19 ^a	0.06 ^a

Means within each row followed by the same letter are not significantly different at P=0.05.

Table 12. The principal constituents of dill fruit oil (mg 100 g fresh weight⁻¹) of the autumn crop in relation to salinity.

Oil composition	R.T. (min)	0.63 dS m ⁻¹	2.0 dS m ⁻¹	4.0 dS m ⁻¹	6.0 dS m ⁻¹
β -myrcene	10.40	0.38 ^a	0.17 ^a	0.37 ^a	nd
α -phellandrene	11.50	1.49 ^b	14.44 ^a	1.27 ^b	1.82 ^b
Limonene	11.89	42.67 ^a	41.35 ^a	39.37 ^a	40.57 ^a
Dill ether	19.50	0.54 ^b	1.03 ^a	1.92 ^a	nd
Cis-dihydro carvone	20.16	0.60 ^a	0.19 ^b	0.28 ^b	nd
Trans-dihydro carvone	21.48	2.30 ^a	2.03 ^a	2.41 ^a	2.34 ^a
Carvone	21.78	50.73 ^a	40.34 ^a	50.57 ^a	54.98 ^a

Means within each row followed by the same letter are not significantly different at P=0.05.

Discussion

Dill is a cool season species and as such grows better in the warm regions of the Mediterranean Basin, (e.g. in Greece) under the relatively low temperatures between autumn and early spring. However, because fresh dill is in demand throughout the year, crops may be grown even in late spring and early summer, but plant growth and the yield of foliage is restricted due to the shorter biological cycle of the plant and the early onset of flowering in response to increasing photoperiod.

In the spring (but rarely in the autumn and winter), plants may be subjected to stress due to water deficiency, which further restricts yield. In many regions too, particularly coastal areas, plants may experience salt stress (salinity) due to sea water contamination of irrigation supplies. Salinity affects crop growth irrespective of the cultivation season, but stress is exacerbated in the spring due to significantly lower precipitation and to higher water evaporation and transpiration as a result of the higher temperatures during this period.

The reduction in plant growth, expressed in terms of plant height, leaf number and plant biomass, as a result of increasing water deficiency in the spring is consistent with the observations of other authors on dill (Mehta et al., 2012; Ghassemi-Golezani et al., 2008), parsley (Petropoulos et al., 2006), oregano and mint (Matraka et al., 2010) and also relates to reduced root length under conditions of water deficiency (Mehr and Ganjeali, 2013). However, it is apparent from the present results that although water stress reduced foliage yield, it did not adversely affect leaf quality. Thus, leaf colour (chlorophyll content) was the same, or even higher, under conditions of water deficiency and the nutritional value (vitamin C and other antioxidants) was the same as in non-stressed plants. Elsewhere, it has been reported that water deficiency may cause a reduction in chlorophyll and carotenoid concentrations (Mehr and Ganjeali, 2013), as observed in mint and oregano (Matraka et al., 2010), but not in *Trachyspermum ammi* (Azhar et al., 2011). These differences, however, may be accounted for by differences in the severity and duration of water stress and other factors (soil, sunlight etc.). For example, Bettaieb et al. (2011) found that the β -carotene and total phenolics content of cumin increased under moderate water deficit, but decreased under severe water deficit. The reduction in the inorganic ion (Na, Cl, K) content of the leaves in response to water deficiency may result from a reduction in root ion uptake efficiency (Mehr and Ganjeali, 2013), but, as shown here, is unlikely to affect leaf quality.

Overall, salinity had a relatively small effect on plant growth, causing a reduction in the number of leaves and plant biomass of the spring crop only at the highest salinity levels (6-8 and 8 dS m⁻¹, respectively), whereas in the autumn both plant height and biomass increased at all levels of salinity. Although dill has previously been recognised as moderately tolerant to salinity, the present results suggest even greater tolerance than this and our data appear to justify the interesting proposal of the Indian Agricultural Research Institute (2014) and Central Soil Salinity Research Institute (Gururaja-Rao et al., 2001b) to cultivate dill (albeit for seed) in saline regions. Moreover, dill appears to be more tolerant to salinity than other aromatic species, such as fennel (*Foeniculum vulgare*) (Abd El-Wahab, 2006). However, salinity may reduce plant height and the number of leaves per plant of dill at an early stage in its growth cycle (up to 30 days from sowing) (Mehr et al., 2012; Mehr, 2013).

The quality of the fresh leaves may be affected by salinity, but the season of growth appears to be a more important factor. Thus plants had a higher chlorophyll content and were therefore greener in the spring, irrespective of salinity. Additionally antioxidants in the leaves (carotenoids, vitamin C and total phenolics) were at higher levels in the spring at nearly all levels of salinity. Overall, there was virtually no effect of salinity on the carotenoid and vitamin C concentrations within the leaves of the autumn crop, while the total phenolics concentrations increased at 6-8 dS m⁻¹. In contrast, in the spring, the vitamin C and total phenolics concentrations decreased with increasing salinity, but the carotenoid concentrations were mostly unaffected. This means that although the nutritional value and appearance (greenness) of fresh dill in the spring was superior to that of the autumn cultivation, the nutritional/antioxidant value decreased with increasing salinity in the spring, but not in the autumn. As in the case of water stress, however, the effect of salinity, both in dill (Parvaiz and Satyawati, 2008; Nourani Azad et al., 2006) and other aromatic species (e.g. fennel) (Mehta et al., 2012), depends not only on the magnitude and duration of the stress, but also on the stage of growth at which stress occurs (Parida and Das, 2005; Abd El-Wahab, 2006).

The concentration of inorganic ions (Na, Cl, K) in the leaves of dill at corresponding levels of salinity was almost invariably higher in the spring than in the autumn. In both seasons the concentrations of Na and Cl in the leaves increased with increasing salinity. However, whereas K also increased with increasing salinity in the spring, in the autumn it fell. An increase in Na and Cl accumulation and a decrease in K in response to increasing applications of NaCl in the irrigation water have been reported for fennel (Ashraf and Akhtar, 2004). In the present experiments, the difference in response of K to salinity in the spring and autumn presumably relates to the climatic conditions during each season: progressively decreasing temperatures and small photoperiod in the autumn (hence less irrigation with saline water), increasing temperatures, photoperiod and light intensity during the spring. However, further experimentation is required to clarify this observation.

The yield of essential oils was higher in the spring than in the autumn. Ghassemi-Golezani et al. (2008, 2011) reported that salinity up to a level of 12 dS m⁻¹ increased the yield of essential oil from leaves, flowers and fruits of dill, but only because of an increase in dry matter. In contrast, Udagawa (1995) reported a decrease in essential oil yield with increasing salinity up to 3.6 dS m⁻¹. It is likely, however, that the difference in the results of these authors stems from the stage at which the plants were harvested. In the present experiments, the plants for herb oil extraction were harvested prior to the onset of flowering and in this case the essential oil was distilled only from leaves. If, however, harvest is delayed and the tissues from which oil is distilled include flowers, or even fruits/seeds, the quantity and composition of oil distilled, as well its the response to salinity, will be different from that derived from leaves alone.

The principal constituents of dill herb oil in the autumn crop were α - and β - phellandrene and dill ether, which is similar to that previously reported (Callan et al., 2007). The characteristic aroma of dill leaves is derived principally from α -phellandrene and dill ether and to a lesser extent limonene and α -pinene (Callan et al., 2007). Qualitative analysis of the essential oils in the leaves showed that α -phellandrene increased in the salinity treatments with 4 and 6 dS m⁻¹, while dill ether decreased. This may cause some change in the aroma profile of the leaves, although this could not be detected macroscopically. Overall, therefore, salinity does not appear to significantly influence either the quantity of oil within the leaves, or its quality, although further research is required to verify these observations. In flower oil, whereas increasing salinity caused a decrease in the relative concentrations of both α -phellandrene and dill ether, there was a concomitant increase in carvone, which is another important oil constituent.

Conclusions

From the results of the present experiments it may be concluded that taking into account both yield and quality aspects, dill is an aromatic plant that is relatively resistant to salinity (up to 6-8 dS m⁻¹) and although growth is less resistant to water deficiency, quality is not impaired. In the warm Mediterranean region, stress due to water deficiency is normally observed only in the spring, whereas stress due to salinity may occur in both the spring and autumn. The quality (chlorophyll and antioxidant content of the leaves) is higher in the spring than in the autumn and the effects of salinity differ between the two seasons.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund. Technical assistance was provided by Andreas Tsirmpas, Georgia Dalmyra, Dimitris Giannoulis and Athanasios Betsis.

References

- Abd El-Wahab, M.A., 2006. The efficiency of using saline and fresh water irrigation as alternating methods of irrigation on the productivity of *Foeniculum vulgare* Mill subsp. vulgare var. vulgare under North Sinai conditions. Res. J. Agric. Biol. Sci. 2 (6), 571-577.
- Adam, P., 1990. Saltmarsh ecology. Cambridge University Press, New York.
- Adams, R.P., 2001. Identification of essential oil components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publ. Corp., Carol Stream, Ill.
- Amirjani, M.R., 2010. Effects of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. Am. J. Physiol. 5, 350-360.
- Arnon, D.I., 1949. Copper enzyme in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 (1), 1-15.
- Ashraf, M., Akhtar, N., 2004. Influence of salt stress on growth, ion accumulation and seed oil content in sweet fennel. Biol. Plant. 48 (3), 461-464.
- Azhar, N., Hussain, B., Ashraf, M.Y., Abbasi, K.Y., 2011. Water stress mediated changes in growth, physiology and secondary metabolites of Desi Ajwain (*Trachyspermum ammi* L.). Pak. J. Bot. 43, 15-19.
- Baher, F.Z., Mirza, M., Ghorbanli, M., Rezaei, M.B., 2002. The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. Flavour Fragrance J. 17, 275-277.
- Bajaj, K.L., Kaur, G., 1981. Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. Analyst. 106 (1), 117-120.
- Bettaieb, I., Knioua, S., Hamrouni, I., Limam, Marzouk, B., 2011. Water deficit impact on fatty acid and essential oil composition and antioxidant activities of cumin (*Cuminum cyminum* L.) aerial parts. J. Agric. Food Chem. 59 (1), 328-334.
- Callan, N.W., Johnson, D.L., Westcott, M.P., Welty, L.E., 2007. Herb and oil composition of dill *Anethum graveolens* L. Effect of crop maturity and plant density. Ind. Crops and Prod. 25 (3), 282-287.
- Charles, D.J., Simon, J.E., Widrelechner, M., 1995. Characterization of essential oil of fruits of dill (*Anethum graveolens* L.). J. Essential Oil Res. 7, 11-20.
- Delfine, S., Loreto, F., Pinell, P., Tognetti, R., Alvino, A., 2005. Isoprenoids content and photosynthetic limitations in rosemary and spearmint plants under water stress. Agric. Eco- systems Environ. 106, 243-252.
- Eaton, A.D., Clesceri, L.S., Greenberg, A.E., 1995. Standard methods for the examination of water and wastewater. 19th ed. Am. Public Health Assoc. Washington. USA.
- Fatima, S.F., Farooqi, A.H.A., Srikant, S., 2000. Effect of drought stress and plant density on growth and essential oil metabolism in citronella java (*Cymbopogon winterianus* Jowitt). J. Med. Arom. Plant Sci. 22 (IB), 563-567.
- Ghassemi-Golezani, K., Andalibi, B., Zehtab-Salmasi, S., Saba, J., 2008. Effects of water stress during vegetative and reproductive stages on seed yield and essential oil content of dill (*Anethum graveolens* L.). J. Food. Agric. Env. 6 (3,4), 282-284.
- Ghassemi-Golezani, K., Zehtab-Salmasi, S., Dastborhan, S., 2011. Changes in essential oil content of dill (*Anethum graveolens*) organs under salinity stress. J. Med. Plants Res. 5 (14), 3142-3145.
- Gururaja Rao, G., Nayak, A.K., Chinchmalatpure, A.R., 2001a. Growth and yield of dill (*Anethum graveolens* L.) on saline black soils of different unirrigated farm sites of Bhal area in Gujarat State. Indian J. Agric. Sci. 71 (11), 712-714.

- Gururaja Rao, O., Nayak, A.K., Chinchmalatpure, A.R., Singh, R., Tyagi, N.K., 2001b. Resource characterisation and management options for salt affected black soils of Agro-Ecological Region V of Gujarat State. Tech. Bull. 112001, Central Soil Salinity Research Institute, Regional Research Station, Anand, Gujarat, India.
- Horneck, D.A., Hanson D., 1998. Determination of potassium and sodium by flame emission spectrophotometry. In: Kalra Y.P. (Ed.), Handbook of Methods for Plant Analysis CRC Press, Boca Raton, FL, USA., pp. 153-156.
- Hughes, S.G., Bryant, J.A., Smironff, N., 1989. Molecular biological application to studies of stress tolerance. In: Hymalyn, G.J., Flowers, T.J., Jones, M.B. (Eds.), Plants under Stress. Cambridge University Press, New York, pp. 131-135.
- Indian Agricultural Research Institute, Zonar Technology Management and Business Planning Development Unit, 2014. Cultivation of dill (*Anethum graveolens*) on saline black soils. <http://www.ztmbpd.iari.res.in/cultivation-dill-anethum-graveolens-saline-black-soils>.
- Jaleel, C.A., Sankara, B., Sridharan, R., Panneerselvam, R., 2008. Soil salinity alters growth, chlorophyll content and secondary metabolite accumulation in *Catharanthus roseus*. Turk. J. Biol. 32, 79-83.
- Jones, H.G., Tardien, F., 1998. Modelling water relations of horticultural crops: a review. Sci. Hort. 74, 21-46.
- Kirk, J.T.O., Allen, R.L., 1965. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. Biochem. Biophys. Res. Commun. 21 (6), 523-530.
- Lisiewska, Z., Kmiecik, W., Korus, A., 2006. Content of vitamin C, carotenoids, chlorophylls and polyphenols in green parts of dill (*Anethum graveolens* L.) depending on plant height. J. Food Comp. Anal. 19 (2,3), 134-140.
- Matraka, M., Ninou, E., Giannakoula, A., Lazari, D., Panou-Filotheou, H., Bosabalidis, A.M., 2010. Effects of soil water content on *Mentha spicata* L. and *Origanum dictamnus* L. Israel J. Plant Sc. 58 (3-4), 229-239.
- Mehr, Z.S., 2013. Salt-induced changes in germination and vegetative stages of *Anethum graveolens* L. J. Stress Phys. Biochem. 9 (2), 189-198.
- Mehr, Z.S., Ganjeali, A., 2013. Effects of drought stress on growth and physiological characteristics of dill (*Anethum graveolens* L.). J. Hort. Sci. (Agric. Sci. Technol.). 27 (1), 27-35.
- Mehr, Z.S., Khajeh, H., Bahabadi, S.E., Sabbagh, S.K., 2012. Changes on proline, phenolic compounds and activity of antioxidant enzymes in *Anethum graveolens* L. under salt stress. Int. J. Agron. Plant Prod. 3, 710-715.
- Mehta, R.S., Anwer, M.M., Sharma, Y.K., 2012. Effect of irrigation, nutrient levels and crop geometry on growth and yield of dill (*Anethum sowa* L.). J. Spices Arom. Crops. 21 (1), 20-24.
- Mostafazadeh-Fard, B., Heidarpour, M., Aghakhani, Q.A., Feizi, M., 2007. Effects of irrigation water salinity and leaching on soil chemical properties in an arid region. Int. J. Agric. Biol. 3, 166-469.
- Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239-250.
- Nourani Azad, H., Ghorbanli, M., Kafilzadeh, F., 2006. The study the effects of NaCl on some physiological traits and chemical composition of dill (*Anethum graveolens* L.). Plant Ecosystem, 2 (8), 48-58.
- Nourani Azad, H., Haji Bagheri, M.R., 2008. Effects of salinity stress on some physiologic characteristics of dill, *Anethum graveolens* L. J. New Agric. Sci. 4 (12), 93-100.
- Ozturk, A., Unlukara, A., Ipekl, A., Gurbuz, B., 2004. Effect of salt stress and water deficit on plant growth and essential oil content of lemon balm (*Melissa officinalis* L.). Pak. J. Bot. 36 (4), 787-792.
- Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effect on plants: a review. Ecotoxicol. Environ. Safety. 60, 324-349.
- Parvaiz, A., Satyawati, S., 2008. Salt stress and phytochemical responses of plants – a review. Plant Soil Environ. 54, 89-99.
- Petropoulos, S.A., Akoumianakis, C.A., Passam, H.C., 2006. Evaluation of turnip-rooted parsley (*Petroselinum crispum* ssp. tuberosum) for root and foliage production under a warm, Mediterranean climate. Sci. Hortic. 109 (3), 282-287.
- Petropoulos, S.A., Daferera, D., Polissiou, M.G., Passam, H.C., 2008. The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. Sci. Hort. 115 (4), 393-397.
- Putievsky, E., Ravid, U., Dudai, N., 1990. The effect of water stress on yield components and essential oils of *Pelargonium graveolens* L. J. Essential. Oil. Res. 2, 111-114.
- Sarker, B.C., Hara, M., Uemura, M., 2005. Proline synthesis, physiological responses and biomass, yield of eggplants during and after repetitive soil moisture stress. Sci. Hort. 103, 387-402.

- Simon, J.E., Reiss-Bubenhiem, D., Joly, R.J., Charles, D.J., 1992. Water stress induced alterations in essential oil content and composition of sweet basil. *J. Essential Oil Res.* 4 (1), 71-75.
- Singh, M., Ramesh, S., 2000. Effect of irrigation and nitrogen on herbage, oil yield and water-use efficiency in rosemary grown under semi-arid tropical conditions. *J. Med. Aromatic Plant Sci.*, 22 (IB), 659-662.
- Udagawa, Y., 1995. Some responses of dill (*Anethum graveolens*) and thyme (*Thymus vulgaris*), grown in hydroponic, to the concentration of nutrient solution. *Acta Hort.* 396, 203-210.
- Zehtab-Salmasi, S., Javanshir, A., Omidbaigi, R., Aly-Ari, H., Ghassemi-Golezani, K., 2001. Effects of water supply and sowing date on performance and essential oil production of anise (*Pimpinella anisum* L.). *Acta. Agron. Hung.* 49 (1), 75-81.
- Zhu, J.K., 2001. Plant salt tolerance. *Trends Plant Sci.* 6, 66-72.