Ratooned fennel relies on osmoregulation and antioxidants to damp seed yield decline with water limitation

Morteza Akbari-Kharaji¹ · Parviz Ehsanzadeh¹ · Ali Gholami Zali¹ · Ehsan Askari¹ · Ahmad Rajabi-Dehnavi¹

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is a medicinal species which has not been examined as a ratoon crop. Ratooning lessens the production intensity, but the ratooned fennel seed yield and physiological processes may differ with stand age, irrigation regime, and genotype. Eleven fennel genotypes were, thus, subjected to either limited or sufficient water supply for 6 years. For each year, the crop was harvested in early autumn to measure seed yield, yield components, dry mass, and essential oil. Besides, some physiological characteristics relevant to seed yield were studied in certain years. Results indicated a biphasic behavior in seed and essential oil yields of the majority of genotypes with maximum seed yields (ranging from 2514 to 2635 kg ha⁻¹ in different genotypes) being obtained from the well-watered plants mainly in the 4th year of harvest. Taking the period of 6 years and the two irrigation regimes together, genotypes Yazd, Kashan, and Shiraz showed a better productive performance. Meaningful positive associations were found between the seed yield and such yield components as seeds/plant and certain physiological attributes, i.e., proline and total soluble carbohydrates concentrations, water potential, relative water content, and superoxide dismutase, catalase, and ascorbate peroxidase activities. Maximum seed yields (ranging from 1605 to 1704 kg ha⁻¹) and essential oil yields (ranging from 33.3 to 45.5 kg ha⁻¹) of plants grown under water-limited conditions were found in genotypes that benefited more from osmoregulative and antioxidative roles of proline and the antioxidative enzymes (i.e., Shiraz, Yazd, and Kashan) and, hence, were able to withstand better against water limitation. Considering such physiological capabilities, agronomic performance, and minimum stand age effects, we discovered for the first time that certain fennel genotypes could be proposed as potential resources for growing fennel as an alternative ratooned crop for low production intensity and sustainable cropping in arid climates.

Keywords Medicinal plant · Essential oil · Water potential · Antioxidative · Seed yield · Perennial

1 Introduction

Ratooning is the cultural practice of cutting crops near their crown after the first harvest of seed or fodder, permitting the crop to grow back for subsequent harvests and, hence, extending production for more than one growing season (Rogé et al. 2016). Thanks to the minimized utilization of agricultural machinery, growing such ratooned crops is thought to lessen the risks of soil degradation and erosion in the already vulnerable arid and semi-arid lands and, in a broader sense, marginal areas occurring typically in the Middle East. In spite of the

definite advantages of growing certain perennial medicinal species, research data about the potential benefits of perennial medicinal and aromatic plants to the sustainability of the agricultural production systems is rare. In effect, most of the available experimental works have focused on the evaluation of seed and essential oil production of medicinal plants over 1–2 years.

While millions of people rely on collecting and processing of medicinal plants either for health care or income from trade of products, there is a fear that many species in high demand will be endangered due to overharvesting (van Andel et al. 2015). Yet, as a consequence of the growing number of consumers and the wider spectrum of the applications of these compounds, the trade of essential oils is going to expand tremendously worldwide in the foreseeable future (Sangwan et al. 2001). Moreover, medicinal plants are generating much interest in arid and semi-arid parts of the world as alternative sources of crop production in less-productive conditions under

✉ Parviz Ehsanzadeh
ehsanzadehp@gmail.com

¹ Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

non-intensive agricultural systems. Continuing a reliance on herbal medicine necessitates conserving these valuable species and hence, in the absence of detailed scientific data, designing appropriate studies on responses of at least commonly traded medicinal plants to environmental factors and agromonic practices. Hence, such necessities have turned the attention of scientists to the medicinal plants (Carrubba and Catalano 2009).

Fennel is an aromatic edible plant from the Umbelliferae (Apiaceae) family and endemic to the Mediterranean conditions, whose mature seeds (Fig. 1a) are used for different purposes since antiquity. Usages in folk medicine, pharmaceutical and cosmetic technology, and production of food preserving compounds in food industries (Diao et al. 2014) are only a few of the main applications of fennel seeds and extracts. Even though it is an herbaceous species that occurs naturally as an annual, it may be cultivated as a perennial crop (Carrubba et al. 2008). An autumn harvest, following retranslocation of nutrients to the crown and bulbous root perennating organs, allows efficient interannual nutrient recycling and spring regrowth (Fig. 1b) in this herbaceous species. Especially interesting is the low-demanding nature and stress-resilient habit of this species as revealed in the previous studies (Gholami Zali and Ehsanzadeh 2018), along

with its different (i.e., compared to ordinary annual crops) production cycle (Carrubba et al. 2008).

Less than 10% of the world arable lands can be stress-free for plants; they are too often faced with an array of constraints, of which drought may rank first in terms of deteriorating plant growth and, thus, agricultural production (Ashraf and Foolad 2007). The unavoidable aftermaths of global climate change, e.g., raised temperatures, altered seasonal weather patterns, increased frequency, intensity and duration of drought episodes, and aggravated scarcity of irrigation water are going to worsen this problem worldwide, in general, and in arid and semi-arid regions, in particular (Kumar et al. 2017). On the other hand, a notable proportion of the world food demand is fulfilled by agricultural production in regions characterized by an inherent water scarcity, e.g., arid and semi-arid parts of the globe. Yet, secondary salinity (e.g., induced by saline irrigation water) aggravates the adverse effect of drought on agricultural plants and endangers food security in arid and semi-arid climates. Plants of less-favorable habitats are expected to have developed adaptation strategies of tolerance to stress factors such as drought. Medicinal plant species occurring in arid and semi-arid regions usually develop adaptive mechanisms for coping with drought stress.

Oxidative stress is known as a secondary phenomenon of environmental stresses imposed on plants, resulting in the generation of the reactive oxygen species (ROS) (Stankovic et al. 2015). Many naturally occurring antioxidant compounds from plant sources are known as reactive oxygen scavengers. Different organs of fennel are potential sources of strong natural antioxidants. Scavenging of ROS in plants exposed to stresses (e.g., drought) is associated with activity of the enzymes involved in antioxidative processes, i.e., superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), the presence of osmoprotectant compounds (such as mannitol, betaine, and proline), and the activity of non-enzymatic antioxidant compounds (such as ascorbic acid, glutathione, thioredoxin, carotenoids, vitamins, and phenolics). While biosynthesis and accumulation of an array of secondary metabolites in plants are considered to be evolutionary response of biochemical pathways to different unfavorable environments such as water deficit, these bioactive compounds may play roles of significance in withstanding stresses (Stankovic et al. 2015). Maintaining internal plant water and turgor potential in a water-limited condition requires an increase in osmotica, normally accomplished by synthesis of metabolically benign solutes, namely organic osmolytes. Accumulation of compatible solutes and osmolytes is known as a means of prolonging plant physiological functions in water-deprived states (LiXin et al. 2009). Though such capabilities have been studied less in the medicinal plants of the fennel type.

To obtain greater essential oil yields and more profitable economics of production of fennel as an alternative cash crop

Fig. 1 **a** Fennel's edible mature seeds are used for different purposes, such as pharmaceutical and cosmetic technology and food industries. **b** Following an autumn harvest, the over-wintered crown and bulbous root perennating organs of fennel allow a spring regrowth

under diverse environmental conditions, our research effort has been focused on developing high-yielding cultivars and physiological modulations of essential oil production under environmental stresses (e.g., water-limited conditions). Current cultivation and production of fennel, at least in the Mediterranean climatic conditions, involves sowing this medicinal species as a winter annual in warmer climates and as a spring annual in cooler areas. This necessitates repeated and, hence, intensive cultural practices in each and every year. Since ratooning minimizes cultural practices and labor costs, we aimed at shedding light on the question of suitability of fennel as a ratoon crop under different soil moisture conditions. In order to arrive at a more appropriate answer to this question, interrelations of seed yield, yield components, and some physiological attributes of fennel were also examined.

2 Materials and methods

2.1 Genetic materials, soil preparation, sowing, irrigation, and maintaining the plants

This 6-year field experiment was carried out at the Lavark Research Farm of Isfahan University of Technology in Najaf Abad (32° 32'N, 51° 23'E, 1630 m above mean sea level), Isfahan, Iran, from 2011 to 2017. Irrigation treatment consisted of irrigation after 35–45% (well-watered) and 75–85% (water-limited) depletion of available soil water (ASW) and the 11 fennel genotypes were “Urmia,” “Hamadan,” “Kerman,” “Shiraz,” “Birjand,” “Yazd,” “Avicenna,” “Kashan,” “Mashhad,” “Bushehr,” and “Isfahan.” The soil was a Fine Loam Typical Haplargid with the initial N, P, K, Zn, and Fe contents of 740.0, 25.0, 225.0, 3.8, and 9.4 mg kg⁻¹, respectively. The soil that had previously been under wheat was tilled and prepared for seeding (consisting of plowing, double-disk harrowing, leveling, and farrowing) in the winter of 2010. The fennel genotypes were obtained from different geographical regions of Iran (hence, were broadly adapted to a wide range of habitats); the seeds were disinfected by a 5% sodium-hypochlorite solution, washed by distilled water, germinated in peat moss and grown to 3–4 leaf stage in plastic trays. Subsequently, the seedlings were transferred to the field in late March 2011. Prior to transferring the seedlings, the plots were irrigated and the seedlings were planted into the irrigated soil. Each plot consisted of five 2-m long farrows spaced 50 cm apart, with seedlings being planted 20 cm apart on each ridge. Hence, fennel plants were planted at the target initial population of 100,000 plants ha⁻¹. Pre-planting fertilizers were avoided as soil analysis did not identify nutrient deficiencies, except for a urea fertilizer (46% N) at 150 kg ha⁻¹. Nitrogen fertilizer was applied each year at growth onset (when plants were nearly 40 cm tall) in late March to early April (with the exception of the establishment

year) as side dressing at 150 kg ha⁻¹ (i.e., urea) before commencing irrigation treatments. The plants were watered two or three times in late winter and early spring each year, then irrigation regimes were applied and continued to approximately 75% physiological maturity, that is, late September of 2011 (i.e., the establishment year) and early to mid-September of the 2012 to 2017. Plots were kept weed-free over the course of the 6-year study by hoeing. No crop diseases and insects were detected during the years 1–4 of the experiment, but in the 5th (2016) and 6th years (2017), application of a Confidor (imidacloprid) insecticide (2.5 ml L⁻¹) deemed necessary for controlling sucking insects such as aphids. Starting from the very first year (i.e., the establishment year), harvests were carried out at the end of each growing season that coincided to mid-September to early October, depending on the year. After fall-winter growth stoppages, the fennels from the previous year regrew, to the extent that a fairly uniform canopy of the stubble crop was achieved around the second half of March each year. While plant height was determined and border plants were not harvested, the outer row in each plot was considered the guard row; plants of a 2-m² area were cut at 7–10 cm above the ground level. A synopsis of temperature and precipitation data was obtained from the nearby Najafabad Weather Station (Fig. 2). The irrigation treatments were applied by calculating ASW, readily available soil water, and the volume of irrigation water and an irrigation water use efficiency of 70% according to Askari and Ehsanzadeh (2015). Irrigations were carried out based on the maximum allowable depletion (MAD) percentage of ASW (i.e., when 35–45% and 75–85% of the ASW were depleted from the root zone for the well-watered and water-limited regimes, respectively). A curve of soil moisture release was created and used to determine the depletion of the ASW based on the soil water potential. Equation (1) was used for determining the irrigation water volume (V_{irrig}) required to rise the water content in the root zone to field capacity.

$$V_{\text{irrig}} = \frac{\text{ASW} \times f}{E_a} \quad (1)$$

In this equation, f is the fraction of ASW (35–45% and 75–85%) that can be depleted from the root zone, and E_a is the irrigation efficiency (%). Irrigation efficiency was assumed to be 70% (Askari and Ehsanzadeh 2015). A synopsis of volume of irrigation water (i.e., applied with a pipe and measured by a flow meter) is as follows: Averaged over the 6 years, well-watered plots received 16.5 irrigations and the inadequately watered plots received 6.5 irrigations per year. In each irrigation event, the well-watered and inadequately watered plots received an amount of 50.6 and 101.3 L m⁻² water, respectively, as these amounts were calculated to be necessary for increasing the root zone soil water content to the field

Fig. 2 A synopsis of mean monthly temperatures and total precipitations over the course of the 6-year long experiment. Data have been obtained from Najafabad Weather Station (32° 63' N, 51° 37' E), Najafabad, Isfahan, central Iran

capacity. Total volume of irrigation water given to the well-watered and inadequately watered plots in each year was 836 and 659 L m⁻², respectively; hence, the inadequately watered plants received nearly 21% less water, compared with the well-watered plants.

2.2 Yield, yield components, and essential oil measurements

Being an indeterminate crop species, the fennel plants were considered mature when 70–80% of the leaves and umbels turned yellowish. Seeds/plant and 1000-seed weights were determined using five plants from the 2nd row of each experimental unit (i.e., plot) at physiological maturity. At around physiological maturity, the plants from the central 2-m² portion in each plot were harvested, air dried for at least 7 days, and seed yield was determined for the 6 years of harvest. Above-ground dry mass (DM) was determined by drying harvested whole plant samples at 72 °C for 72 h in the aforementioned years. The harvest index was determined as the ratio of seed weight to DM. Moreover, a 20-g sample of seeds of each plot was grinded to a powder and 200 mL of deionized water was used for carrying out the hydrodistillation process on the

essential oil at 100 °C (Askari and Ehsanzadeh 2015). The essential oil phase was separated, dried over anhydrous sodium sulfate, and essential oil concentration and yield were expressed as percent and kilograms per hectare, respectively.

2.3 Leaf water relations and osmolytes measurements

As the main purpose of this study was to elucidate agronomic attributes of fennel as a ratooned crop, assessment of the physiological attributes was limited to the final year of the study (i.e., the 6th year). At the 50–70% flowering stage, leaf water potential, relative water content (RWC), proline, and total soluble carbohydrate (TSC) concentrations of plants in each experimental unit were measured in early June of the 6th year (i.e., 2017). Mid-day water potential was determined as described in Askari and Ehsanzadeh (2015) using a portable pressure chamber instrument (*PMS Model 600*, USA). The mean of two measurements was calculated for each plot. RWC was measured on leaf sections by measuring turgid, fresh, and dry masses according to the procedure explained in the aforementioned study. Free proline content of the leaves was measured using 200 mg of fresh mature leaves that had been crushed in sulphosalicylic acid and added into a

ninhydrin reagent and assessed according to spectrophotometric absorbance at 520 nm (*Hitachi U1800, Japan*), as detailed in Askari and Ehsanzadeh (2015). Leaf TSC was measured using 500 mg of homogenized samples of dried leaves that had been ethanol-extracted and mixed with anthrone and subjected to spectrophotometric measurements at 625 nm according to the details given in the latter study.

2.4 Antioxidative enzymes activity and carotenoids measurements

The concentration of carotenoids in fresh leaves was measured using 500 mg of fresh leaf tissue that had been taken at 70% flowering of the 1st and 2nd years, crushed in a 80% acetone solution, extracted and subjected to the spectrophotometer for measuring absorbencies according to the details given by Askari and Ehsanzadeh (2015).

In order to measure antioxidant enzymes activities, 500 mg of leaf samples that had been obtained around the 50–70% flowering stage were homogenized in an extraction buffer, centrifuged at 15,000 rpm for 20 min at 4 °C and enzyme activities were expressed according to the measured total protein content of the samples. APX activity was measured spectrophotometrically by a decrease in the absorbance at 265 nm according to method described by Gholami Zali and Ehsanzadeh (2018). CAT activity was measured by measuring consumption of H₂O₂ using absorption by spectrophotometer at 240 nm according to the details given in the aforementioned study. The activity of SOD was assayed by measuring ability to inhibit photoreduction of nitro blue tetrazolium (NBT) using the procedure described by Askari and Ehsanzadeh (2015).

2.5 Statistical analysis

The experimental design was a split-plot randomized complete block with three replications. Over the course of the 6 years of this study, a plot was considered to be the experimental unit and all statistical analyses and calculations were carried out on the plot mean. A mixed model repeated-measures ANOVA was employed to evaluate differences in yield and yield components between genotypes across the entire study period (i.e., 6 years of harvest). Genotype and irrigation were treated as fixed categorical variables, and year was specified as a repeated-measure term with a plot as the experimental unit. Albeit, leaf water relations, osmolytes concentration, and antioxidative activities were measured only in certain years, these data were subjected to a simple analysis of variances. Tukey's Honestly Significant Difference (HSD's) procedure was employed to conduct mean comparisons.

3 Results and discussion

3.1 Agronomic and seed essential oil attributes over the course of 6 years

The two levels of the irrigation regime (with the exception of the harvest index), the 11 fennel genotypes, and the 6 years of harvest were found to bear significant differences in terms of seed yield, DM, harvest index, 1000-seeds weight, seeds/plant, plant height, essential oil concentration, and essential oil yield (Table 1). Furthermore, genotype $\times$ irrigation, year $\times$ irrigation, year $\times$ genotype, and genotype $\times$ irrigation $\times$ year interaction effects were found to be significant for all of the aforementioned traits, except for the 1000-seed weight, where the genotype $\times$ irrigation interaction was not found statistically significant and for the plant DM, where year $\times$ irrigation interaction did not appear to be statistically significant (Table 1). Hence, due to the significant effects found with the mentioned interactions, mean comparisons will be restricted to the second order interactions hereafter (i.e., genotype $\times$ irrigation $\times$ year), unless otherwise emphasized.

3.1.1 Seed yield, yield components, and plant dry mass

The majority of the genotypes tended to produce a greater seed yield either in the 2nd, 4th, or 5th years of harvest under the well-watered condition. Albeit, the inadequately watered fennel plants for the majority of genotypes indicated greater seed yields in the 4th year and in a lesser extent in the 3rd and 5th years of harvest (Table 1). With the exception of a few genotypes that tended to produce a greater seeds/plant in the 2nd year, the remaining genotypes appeared to produce the greatest seeds/plant in the 4th and 5th years of harvest both in the presence and absence of water limitation. The greater seed yields of certain genotypes, exemplified by Kashan, Yazd, and Shiraz, were accompanied by greater seeds/plant of these genotypes in most years. Meanwhile, well-watered and inadequately watered 2- and 3-year-old fennel plants produced the greatest 1000-seed weight; albeit there were a few exceptions, where 1- and 6-year-old plants produced the greatest 1000-seed weight. The greatest plant height and, hence, DM (Table 2) of well-watered and inadequately watered plants for most of the genotypes were found in the 5th and 4th, and in a lesser extent 3rd years of harvest.

At present, no long-term continuous yield study is available in the peer-reviewed literature, making an accurate assessment of the yield trend of fennel much difficult. The main source of economic value associated with using fennel as a medicinal crop is its seeds, from which the maximum amount of essential oil can be extracted (Gholami Zali and Ehsanzadeh 2018). The majority of fennel genotypes showed a similar productivity trend characterized by two seed-yielding phases: an increasing phase from the 1st to the 4th year and a decreasing

Table 1 Means of interactions of genotype $\times$ irrigation regime $\times$ year and a synopsis of analysis of variances

Fennel genotype	Irrigation regime	Seed yield (kg ha ⁻¹)						Seeds/plant									
		Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6				
Urmia	W-watered	1343	1858	1199	1725	1596	1477	3850	5167	3238	5346	7038	5153				
	Water-L	459	683	1080	1156	885	269	1636	2380	3286	6409	4802	949				
Hamadan	W-watered	1168	1608	1478	1068	1556	1215	3436	4443	3962	3955	7199	3860				
	Water-L	291	443	655	616	591	161	1236	1801	1880	2876	3285	580				
Kerman	W-watered	543	781	825	2214	1585	1486	2017	2760	2477	7938	7492	5654				
	Water-L	336	488	601	1370	889	249	1573	2062	1964	7876	5217	1179				
Shiraz	W-watered	1460	1874	815	1861	1811	1315	4607	5512	2221	7369	9137	4686				
	Water-L	776	1078	713	1206	901	716	2921	3880	2163	5676	4971	2781				
Birjand	W-watered	1120	1661	2010	2247	1920	1374	3509	4966	5668	7409	8002	4804				
	Water-L	321	459	579	904	791	483	1418	1910	1887	3781	3984	1832				
Yazd	W-watered	1080	1429	2478	2007	2177	1686	3205	4269	7100	7145	8033	5785				
	Water-L	748	1079	753	1605	542	616	2690	3855	2442	8676	2874	2272				
Avicenna	W-watered	1539	2203	1175	2397	2445	1289	4683	6130	2965	8080	10,896	4002				
	Water-L	299	621	642	1113	899	499	1263	2425	1772	4796	4029	1836				
Kashan	W-watered	1309	1679	2291	2635	2342	1563	3967	4885	5616	9512	10,361	5845				
	Water-L	529	686	1089	1704	623	489	2037	2535	2824	8268	2924	2136				
Mashhad	W-watered	1043	1650	1199	2514	1205	1026	2627	4241	3132	8891	4193	3393				
	Water-L	461	775	713	1028	800	174	1474	2581	2008	4097	4021	662				
Isfahan	W-watered	1526	2110	1723	1865	3319	1755	4355	5556	4107	5962	13,488	6328				
	Water-L	490	688	913	762	856	278	1846	2457	2804	3606	3944	1138				
Bushehr	W-watered	1213	1881	1154	1132	1842	1028	3338	4990	3394	4067	6992	3917				
	Water-L	413	708	734	867	763	481	1522	2471	2346	4213	3580	1816				
Tukey's HSD _{5%}		123	1000-seeds weight (g)						529	Plant height (cm)							
Urmia	W-watered	3.50	3.60	3.71	3.20	2.27	2.84	93	100	154	177	191	153				
	Water-L	2.80	2.87	3.30	1.83	1.90	2.84	80	77	137	139	160	112				
Hamadan	W-watered	3.40	3.63	3.73	2.70	2.20	3.14	93	138	176	172	176	141				
	Water-L	2.37	2.47	3.50	2.17	1.83	2.78	87	30	137	126	135	108				
Kerman	W-watered	2.70	2.87	3.33	2.77	2.10	2.65	65	85	172	179	176	136				
	Water-L	2.17	2.37	3.07	1.83	1.70	2.20	57	55	147	143	157	109				
Shiraz	W-watered	3.17	3.40	3.68	2.53	2.00	2.82	95	125	186	193	183	135				
	Water-L	2.67	2.77	3.32	2.13	1.80	2.60	82	85	138	127	145	119				
Birjand	W-watered	3.20	3.37	3.55	3.07	2.53	2.81	93	123	175	187	177	139				
	Water-L	2.27	2.40	3.07	2.40	1.97	2.65	67	25	152	140	150	117				
Yazd	W-watered	3.37	3.33	3.50	2.80	2.70	2.92	93	92	173	162	178	132				
	Water-L	2.80	2.80	3.10	1.83	1.90	2.72	68	57	121	111	118	112				
Avicenna	W-watered	3.30	3.60	3.97	3.10	2.27	3.22	110	143	186	185	186	150				
	Water-L	2.37	2.57	3.63	2.30	2.23	2.72	87	53	138	141	142	121				
Kashan	W-watered	3.30	3.43	4.08	2.77	2.27	2.71	88	115	169	156	183	121				
	Water-L	2.60	2.70	3.87	2.10	2.13	2.29	78	78	132	126	141	121				
Mashhad	W-watered	3.97	3.90	3.83	2.83	2.87	3.01	87	115	172	181	184	154				
	Water-L	3.13	3.00	3.57	2.47	2.00	2.64	80	60	121	107	127	124				
Isfahan	W-watered	3.50	3.80	4.20	3.10	2.50	2.77	90	145	174	167	182	159				
	Water-L	2.67	2.80	3.33	2.13	2.20	2.44	82	65	137	135	156	105				
Bushehr	W-watered	3.63	3.77	3.40	2.77	2.67	2.67	80	127	168	159	184	140				
	Water-L	2.73	2.87	3.13	2.10	2.17	2.64	70	60	123	114	131	109				
Tukey's HSD _{5%}		0.11							12.75								
A synopsis of analysis of variances																	
	Seeds/plant	1000-S	H	DM	SY	HI	EOC	EOY	Carotenoids	CAT	APX	SOD	Proline	TSC	RWC	WP	
Irrigation (I)	**	**	**	**	**	ns	**	**	**	**	**	**	**	**	**	**	
Genotype (G)	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	
Year (Y)	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	
G × I	**	ns	**	**	**	**	**	**	**	**	**	**	**	ns	ns	**	
Y × I	**	**	**	ns	**	**	**	**	**	**	**	**	**	**	**	**	
Y × G	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	
Y × I × G	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	

For each trait means with differences greater than Tukey's HSD value are not significantly different at 0.05 level. **, significant at $p \leq 0.01$; ns, non-significant at $p \leq 0.05$; W-watered, well-watered; Water-L, water-limited; 1000-S, 1000 seeds weight; H, plant height; DM, plant above-ground dry mass; SY, seed yield; HI, harvest index; EOC, essential oil concentration; EOY, essential oil yield; CAT, catalase; APX, ascorbate peroxidase; SOD, superoxide dismutase; TSC, total soluble carbohydrates; RWC, relative water content; WP, water potential

phase related to the 5th and 6th years of growth (Table 1). Evidently, seed yields of the majority of the examined

genotypes were decreased with stand ages beyond the 4th year. However, this decline was proportionately smaller or

occurred after the 5th year in certain genotypes, suggesting a weaker effect of stand age on these genotypes. Overall, the greatest fennel seed yield under well-watered and inadequately watered conditions was obtained from genotypes that were less affected by stand age (i.e., Yazd followed by Kashan, Shiraz, and Isfahan). Precipitations in April–May, when a closed crop canopy had almost been attained, were greater in the 5th and 6th years of harvest, compared with the remaining years. This may account for a tendency to greater plant height, DM, and hence seed yield in the 5th year for a number of the genotypes. However, since such tendency was not greater in the inadequately watered plants, compared with the well-watered plants, this proposition was ruled out. Both biphasic pattern of the seed yield and its decline with stand age are generally observed in perennial herbaceous crop species, e.g., *Miscanthus × giganteus* and *Panicum virgatum* (Arundale et al. 2014) and *Arundo donax* (Angelina et al. 2009). Among the studied fennel seed yield components, 1000-seed weight and seeds/plant were the least and the most variable attributes, respectively, across irrigation regimes and years (Table 1). Since the greatest seed yields were observed in genotypes with the greatest seeds/plant, it is reasonable to surmise that seeds/plant (unlike 1000-seed weight) was the main contributor to the variations revealed in the seed yield of fennel. This finding conforms to the notion that seed yield of crop plants is mainly tuned by the number of seeds, rather than the individual seed weight (Gholami Zali and Ehsanzadeh 2018). A negative correlation between kernel number per wheat spike and kernel size has been emphasized (Würschum et al. 2018). Furthermore, among all yield components of common bean, seed size (and hence 1000-seed weight) indicated the smallest modifications across different years and locations when plants were grown under drought condition (Assefa et al. 2015). We did not attempt to measure photosynthesis, but a supposedly maintained photosynthesis might have helped fennel to yield higher seeds/plant through lowering flower infertility. Our data share commonalities with those of Carrubba et al. (2008), who reported DM (Table 2) and seed yields of biennial fennel to be around 7000 and 500 kg ha⁻¹, respectively. Moreover, our findings agree to those of Özel et al. (2019), who reported the maximum seed yield of a fennel genotype to range from 1580 to 1785 kg ha⁻¹ over the course of two consecutive years. This report has confirmed that fennel seed yield may, also, be affected by plant population density. Even though fennel genotypes in our study were initially established in a given plant population density (i.e., 10 plants m⁻²), differential age-associated modifications in the seed yield of the genotypes might be attributed to changes in population densities during the 6-year study period. The proposed age effects might have soared in the aftermath of the water limitation, leading to substantial decreases in the seed yield of several fennel genotypes in the 5th and 6th years of harvest. Another shared feature of our

results and those of Carrubba et al. (2008) is plant height, where they reported that fennel plant height ranged from around 130 to 150 cm. Drought-induced decreases in growth attributes and plant DM, along with increases in essential oil concentration have been reported for a number of other medicinal plants, including *Salvia officinalis* (Bettaieb et al. 2009) and *Origanum vulgare* (Azizi et al. 2009). Moreover, significant decreases in seed yield and yield components of *Carum carvi* under drought conditions have been noted (Laribi et al. 2009).

3.1.2 Harvest index and seed essential oil

Except for a few cases where well-watered plants indicated greater harvest indices in the 6th year, the majority of the genotypes under the water-limited and well-watered conditions indicated the highest harvest indices in the 2nd and to a lesser extent 1st years of harvest (Table 1). While Kashan, Yazd, Shiraz, and Isfahan out-numbered the remaining genotypes under water-limited conditions in terms of the harvest index in several years, genotypes Urmia, Isfahan, and Hamadan indicated greater harvest indices under well-watered conditions (Table 2).

The harvest index, as a measure of plant photoassimilates partitioning, is a reflection of the fraction of the total DM allocated to the seed and maintaining a high harvest index has been proposed as a suitable strategy for improving crop yield under water-limited conditions. In the present study the higher-yielding fennel genotypes (i.e., Yazd, Kashan, and Shiraz) out-numbered the remaining genotypes in terms of the harvest index at least when grown under water-limited conditions (Table 2). In agreement with our findings, high-yielding red common bean genotypes have showed greater harvest index values under drought conditions, leading to a consistent positive correlation between seed yield and harvest index in the presence of stress (Assefa et al. 2015). Given the fact that many medicinal species, including fennel (in contrast to most cultivated crops), have not been submitted to a long lasting genetic improvement process (Carrubba and Militello 2013), the finding that all of the examined genotypes featured a low harvest index across all years and the two irrigation regimes (Table 2) did not come as a surprise. A tendency towards harvest index stability has been confirmed for different crop species, ranging from cereals such as maize (Echarte and Andrade 2003) and barley (Ingvordsen et al. 2015) to legumes such as common bean (Assefa et al. 2015). However, since excessive vegetative regrowth has been emphasized as one of the drawbacks of the ratooning in the literature (Rogé et al. 2016), the tendency of the fennel genotypes to decreasing harvest indices with the harvest year (i.e., stand age) was not far from our expectation.

The tendency to a greater seed essential oil concentration in the 5th and 6th years of harvest was seen in the inadequately

Table 2 Means of interactions of genotype $\times$ irrigation regime $\times$ year for seed yield, harvest index, seed essential oil concentration, and essential oil yield of fennel

Fennel genotype	Irrigation regime	Plant above-ground dry mass (ton ha ⁻¹)						Harvest index (%)					
		Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Urmia	W-watered	5.37	8.27	17.97	17.33	20.04	9.33	25.0	22.7	6.68	10.5	8.18	16.2
	Water-L	2.03	2.31	17.00	12.95	17.80	2.70	22.9	29.4	6.37	8.94	5.01	9.55
Hamadan	W-watered	5.10	6.43	14.23	14.10	17.12	7.78	23.2	25.6	10.4	8.02	9.27	16.1
	Water-L	1.65	2.34	13.28	10.37	14.35	1.44	17.8	18.9	4.93	6.04	4.01	11.8
Kerman	W-watered	4.88	4.93	15.77	17.87	21.12	9.19	10.9	15.7	5.24	12.5	7.57	15.9
	Water-L	3.08	2.71	7.67	20.80	16.35	4.55	11.2	18.3	7.84	8.16	5.43	5.16
Shiraz	W-watered	6.58	9.57	18.96	21.21	22.23	9.09	22.6	19.9	4.30	8.84	8.22	14.6
	Water-L	2.46	5.04	9.27	11.07	16.61	5.64	31.0	21.0	7.69	11.4	5.78	14.0
Birjand	W-watered	6.14	7.99	12.81	16.28	18.05	7.93	18.9	20.7	15.7	14.1	10.7	16.9
	Water-L	1.61	2.40	11.69	11.00	11.73	3.25	20.2	19.2	4.95	8.63	6.73	15.0
Yazd	W-watered	5.17	7.37	26.41	14.88	20.60	7.31	21.9	19.5	9.39	13.5	10.6	23.0
	Water-L	2.48	3.62	5.05	12.00	9.29	4.30	30.9	30.1	15.0	13.4	6.08	13.7
Avicenna	W-watered	7.41	11.84	26.54	26.11	22.51	8.05	21.3	18.7	4.43	9.36	10.9	16.2
	Water-L	1.63	3.03	12.29	17.95	12.32	3.66	18.5	20.7	5.23	6.63	7.77	13.9
Kashan	W-watered	7.55	8.79	21.04	17.62	17.67	8.66	17.4	19.3	10.9	15.6	13.4	18.5
	Water-L	1.57	3.47	17.75	11.82	11.78	4.06	34.1	19.9	6.20	14.5	5.33	12.6
Mashhad	W-watered	8.86	9.05	13.80	19.23	17.13	7.91	11.8	18.8	8.70	13.6	7.05	14.3
	Water-L	2.64	3.87	11.42	12.04	15.13	2.37	17.5	20.4	6.25	8.40	5.34	7.32
Isfahan	W-watered	7.25	9.34	11.24	20.60	22.68	8.36	21.3	23.0	15.4	9.02	14.6	21.5
	Water-L	1.56	3.13	9.23	14.97	17.06	2.14	32.1	22.1	10.1	5.37	5.01	13.1
Bushehr	W-watered	6.70	11.88	20.82	17.10	25.91	11.18	18.3	16.3	5.54	7.47	7.39	9.17
	Water-L	2.19	3.16	7.37	13.77	12.79	5.69	18.7	22.6	10.0	7.47	6.04	9.01
Tukey's HSD _{5%}		1.30						1.78					
		Essential oil concentration (%)						Essential oil yield (kg ha ⁻¹)					
Urmia	W-watered	1.69	2.44	1.94	2.61	3.92	3.30	22.7	45.1	23.2	45.0	62.5	48.7
	Water-L	1.99	2.79	2.16	4.41	5.56	3.45	9.10	18.8	23.4	51.3	49.2	9.30
Hamadan	W-watered	1.95	2.05	2.01	2.38	1.82	2.85	22.7	33.0	29.7	25.3	28.4	34.8
	Water-L	1.79	1.81	2.03	2.88	3.95	3.92	5.20	7.90	13.3	18.3	24.0	6.00
Kerman	W-watered	1.71	1.93	1.80	2.87	2.54	2.85	9.10	14.6	14.8	63.7	40.0	43.3
	Water-L	1.93	2.31	2.24	2.70	3.37	3.57	6.50	11.4	13.5	37.7	30.2	8.50
Shiraz	W-watered	1.75	2.29	1.79	2.58	2.26	3.47	25.5	43.0	14.6	48.7	40.9	45.7
	Water-L	2.07	2.63	2.51	2.34	3.33	4.83	15.7	27.4	18.0	28.3	29.8	34.4
Birjand	W-watered	1.85	1.96	1.91	3.24	3.27	2.98	20.9	31.9	38.4	72.8	62.7	40.3
	Water-L	1.77	1.92	1.92	3.24	4.12	3.75	5.70	8.80	11.1	29.0	32.4	18.8
Yazd	W-watered	1.82	2.36	2.24	3.30	2.56	2.90	20.0	33.2	55.5	67.0	55.4	48.8
	Water-L	1.95	2.51	2.37	2.80	4.55	3.58	14.6	27.1	17.8	46.0	24.6	22.0
Avicenna	W-watered	1.92	2.55	2.18	3.06	2.97	2.93	29.7	55.7	25.7	73.3	72.7	38.1
	Water-L	1.77	2.13	2.08	3.25	3.52	3.92	5.30	13.2	13.4	36.3	31.6	19.5
Kashan	W-watered	1.68	1.99	2.06	3.58	2.85	3.65	21.8	33.2	47.3	94.7	66.7	58.5
	Water-L	1.82	2.01	2.06	3.05	4.25	4.23	9.60	13.8	22.3	54.0	26.6	20.7
Mashhad	W-watered	1.97	2.11	2.07	3.67	2.95	3.22	20.3	35.2	24.9	91.3	35.5	33.3
	Water-L	2.31	2.31	2.39	2.71	3.42	3.53	10.5	18.0	17.1	29.3	27.4	6.20
Isfahan	W-watered	1.89	1.98	1.96	2.87	2.46	3.02	28.7	41.7	33.7	54.0	81.6	53.2
	Water-L	2.01	2.38	2.25	2.84	3.55	3.82	9.90	16.3	20.9	21.7	30.8	10.6
Bushehr	W-watered	1.82	1.93	1.94	3.10	2.56	3.18	22.0	36.8	22.3	35.7	47.6	32.7
	Water-L	1.87	1.96	1.93	2.80	4.03	3.97	7.60	13.9	14.1	24.7	30.6	19.1
Tukey's HSD _{5%}		0.17						4.5					

W-watered, well-watered; Water-L, water-limited. For each trait means with differences greater than Tukey's HSD value are not significantly different at 0.05 level

watered plants but that of the well-watered plants was observed in the 4th year of harvest (Table 2). Even though essential oil concentration of the majority of the examined fennel genotypes increased under the water-limited condition in most harvest years, essential oil yield of all examined genotypes (with the exception of genotype Urmia at the 4th year) decreased due to water limitation across all years. Consistent with seeds/plant and seed yield, most of the fennel genotypes produced the greatest essential oil yield in the 4th and 5th years of harvest for both well-watered and water-limited

conditions (Table 2). Well-watered plants of genotypes Kashan and Isfahan numbered out the well-watered plants of other genotypes, though inadequately watered plants of Urmia, Shiraz, Kashan, and Yazd out-numbered the inadequately watered plants of remaining genotypes in terms of essential oil yield in most of the years of harvest.

Variation in fennel seed's essential oil concentration over the 6-year field study period and among the examined genotypes and irrigation regimes (e.g., 1.69 to 5.56% in Table 2) did not come as a surprise, as decreases in essential oil content

with increase in the degree of fennel seed's maturation have been established; it may vary considerably with ecological and environmental conditions under which the plants have been grown and the seeds have matured (Telci et al. 2009). Essential oil production is not independent from the physiology of the whole plant, i.e., it depends on the metabolic state and background conditions under which the synthesizing tissue has undergone developmental program (Sangwan et al. 2001). This trait may vary with ontogeny, seasonal, and climatic variations (e.g., soil moisture, salinity, and prevalent temperature), mineral nutrition status, light quantity and quality, and duration. Being located in the center of the arid zone of Central Iran, total annual precipitation (starting from October and ending in September) over the course of the study period ranged from nearly 47 mm in 2017 to 217 mm in 2012 and the precipitations were mainly concentrated in late autumn to mid spring, but the pattern of its monthly and seasonal distribution varied considerably from year to year (Fig. 2). Mean monthly temperatures experienced varied from around 3–8 °C (in January) to 31–33 °C (in June and July), but some extreme temperatures were recorded over the 6-year study period. The latter variations in seasonal and monthly temperatures and precipitations may have affected the essential oil synthesis process and hence its concentration in different years. Moreover, the presumption of suitability of genotypes Yazd, Shiraz, and Kashan for a more profitable fennel seed production (compared with the remaining of the examined genotypes) is further substantiated as the greatest essential oil yields were also found for the genotypes Yazd, Kashan, Isfahan, Mashhad, and Birjand in some harvest years.

3.2 Osmolytes and water relation attributes

Analysis of variances of the traits related to osmoticums and water relations of the plants in the 6th year of harvest indicated that all attributes (i.e., leaf proline and TSC concentrations, RWC, and water potential) were significantly affected by the irrigation regimes and genotypes (Table 1). Furthermore, leaf proline concentration and water potential were significantly affected by the genotype $\times$ irrigation interaction. Mean TSC was increased by 17% and mean RWC was decreased by 19% when fennel plants were inadequately watered in the 6th year of harvest (data not shown). Furthermore, the greatest means of the aforementioned attributes, particularly under water-limited conditions, were recorded for a set of genotypes characterized by greater seed yields in the presence of water limitation (e.g., Kashan, Yazd, Isfahan, and Shiraz). Leaf proline concentrations were increased (Fig. 3e) and water potentials (Fig. 3f) were decreased for all genotypes (albeit in different extents) when grown under water-limited conditions, compared with the well-watered conditions.

Stress-triggered proline accumulation could be due to its de novo synthesis, lowered degradation, and depressed utilization,

or enhanced hydrolysis of proteins (Kaur and Asthir 2015). During osmotic stress episodes, the proline degradation pathway is downregulated, bringing about free proline accumulation. It has been established that proline catabolism is blocked in the root tips of sensitive genotypes under severely stressed conditions (Kavi Kishor and Sreenivasulu 2014). Upon the relief of the stress, however, growth is enhanced through reliance on the proline reservoir. The osmoprotecting role of the proline is associated with maintaining the leaf water status, as exemplified by maintained leaf water potential (Fig. 3f) and RWC (data not shown) for the examined fennel genotypes in the present study. Fennel genotypes with high proline and TSC accumulation capabilities (i.e., Yazd, Kashan, Shiraz, and Isfahan) were among those with the greatest RWC and leaf water potential, a finding that has been almost confirmed in previous studies (Gholami Zali and Ehsanzadeh 2018). Genotypic and species differences in the degrees and types of solutes accumulated, and thus osmotic adjustment capacities have been recognized in many plant species (Zhang et al. 1999; Kumar et al. 2017). The water deficiency-induced increases in proline and TSC concentrations (74% and 17%, respectively) in fennel plants may have been brought about by enhanced biosynthesis, depressed degradation, or altered inter- and intra-cellular transport of these osmolytes. Our results are in line with those of Kumar et al. (2017), in that they found soluble sugars as major contributors to oleander (*Nerium oleander* L.) resistance to both drought and salt stresses. Patterns of RWC and water potential modifications in fennel genotypes in response to irrigation water availability bore an intimate resemblance, which is in accord with the resemblance of these attributes reported by Fernandes-Silva et al. (2016) in their work on drought-stricken olives. Moreover, proline is a multifunctional molecule that is able to prevent cellular damage by acting both as an osmotic agent and a radical (i.e., ROS) scavenger (Gholami Zali and Ehsanzadeh 2018). It must be emphasized that proline is not alone in tackling ROS-induced oxidative stresses. As it is discussed hereinafter, ROS abundance is often controlled by an associated increase in non-enzymatic and enzymatic antioxidants. Our data indicated that TSC- and proline-mediated osmotic adjustment has played a major role in productivity maintenance under water-limited conditions and supported the idea that osmotic adjustment sustains crop yield under drought stress (Blum 2017). We did not undertake direct osmotic adjustment measurements, but we may surmise that these genotypes differed in osmotic adjustment capability, based on the notable differences in RWC and water potential (Fig. 3f) sustainment along with TSC and proline accumulation (Fig. 3e) of the examined fennel genotypes. Furthermore, based on the proportionality between the osmolytes accumulation, RWC, water potential, and seed and DM sustainment, it may be inferred that certain genotypes (i.e., Yazd, Kashan, Shiraz, and Isfahan) outperformed the remaining genotypes not only in terms of undergoing osmotic adjustment but also with respect to seed yield under water-limited conditions. Generally, it is not

Fig. 3 Means of genotype × irrigation regime interactions for carotenoids concentration (a), catalase (CAT) (b), ascorbate peroxidase (APX) (c), superoxide dismutase (SOD) activities (d), proline concentration (e), and water potential of fennel (f). Carotenoids concentration, CAT, APX,

and SOD activities are means of data obtained from measurements in 2011 and 2012. Proline concentration and water potential have been measured on plant leaves in 2017. Bars are indicative of standard error

straightforward to find a singular plant trait responsible for the yield advantage of a crop under water-limited condition. The presented data herein, however, depicts the less-studied fennel as a crop that resembles well-attended crops such as barley, wheat, maize, sorghum, chickpea, pea, pigeon pea, soybean, canola, mustard, castor bean, and sunflower (Blum 2017) in that a positive and clear association exists between osmotic adjustment and yield under water-limited conditions. These osmotic adjustment measures have, presumably, helped the inadequately watered fennel plants to sustain the photosynthetic activity and provide photoassimilates to lessen flower infertility and, consequently, maintain seeds/plant and seed yield.

3.3 Antioxidative activities

Statistical analyses of the antioxidative activities revealed that carotenoids concentration and CAT, SOD, and APX activities were significantly affected by irrigation regime, genotype, and genotype × irrigation interaction (Table 1). Carotenoids concentration responded to irrigation regime in a genotype-specific manner, i.e., while it increased with water limitation for half of the genotypes, it decreased under water-limited condition in the

other half, in comparison with the well-watered condition (Fig. 3a). CAT (Fig. 3b) and APX (Fig. 3c) activities responded to water limitation in genotype-specific manners. In effect, while in certain genotypes, no notable changes were observed, in the remaining genotypes, significant increases in the activities of the latter enzymes were observed when plants were grown under water-limited conditions. SOD activities were increased substantially (these increases varied from 100% to 283% among the genotypes) for all examined genotypes in response to water limitation (Fig. 3d). The greatest activities of this set of three enzymes in the inadequately watered plants were found in the genotypes Yazd, Kerman, and Shiraz and the greatest means for carotenoids concentrations were detected in the genotypes Yazd, Mashhad, and Bushehr.

Almost all plant species withstand drought stress through relying on a myriad of mechanisms, but the extent of tolerance and the mechanism relied on vary from species to species. A wide range of stresses, including drought, trigger a plethora of antioxidative systems to circumvent the potentially harmful effects caused by stress-induced generation of ROS (Reddy et al. 2004). Stomatal limitation aside, drought induces decreases in photosynthesis through metabolic impairments, due to an

imbalance between light capture and its utilization in photosystem II (PSII), often leading to a boost in the ROS generation. The latter phenomenon per se necessitates triggering antioxidative machinery. While SOD, CAT, and APX can be categorized into the enzymatic type, carotenoids are among the non-enzymatic types of the antioxidative system. All of the aforementioned antioxidative defense components were more or less enhanced when fennel plants were exposed to water limitation (Fig. 3a–d). However, the magnitude of the increase in the activities of antioxidant enzymes and the amount of antioxidants are extremely variable among species, genotypes of the same species, different development stages and physiological states of the same plant, and more importantly with duration and intensity of the stress (Reddy et al. 2004). Therefore, a certain variation in the level of expression of these antioxidants in the fennel genotypes did not come as a surprise. A higher antioxidant capacity and, hence, tolerance to drought is often associated with increases in the expression and activity of CAT, SOD, and APX (Zandalinas et al. 2018). Moreover, drought-high light stress tolerance of the aridity-adapted cashew (*Anacardium occidentale*) plants has been featured by enhancements in the activities of SOD and APX (Lima et al. 2018), which confirms our findings. Hence, correlative associations between the activity of these enzymes and seed yield and DM sustainment in the presence of stress are established. Evidence for such associations was obtained in the present study, where genotypes that were capable of enhancing SOD, CAT, and APX activities (e.g., Yazd and Shiraz) and maintaining carotenoids concentration (e.g., Yazd, Bushehr, and Mashhad) were among the high-yielding ones, particularly under water-limited condition. Based on the notable accumulation of osmolytes and upregulation of antioxidative enzymes, it may be concluded that key to the partially maintained yields of inadequately watered plants of certain fennel genotypes (e.g., Yazd, Shiraz, and Kashan) lies in their osmotic adjustment capability accompanied by an efficient antioxidative defensive capacity. Maintaining leaf water and antioxidative defense has, perhaps, enabled inadequately watered Yazd, Shiraz, and Kashan plants to sustain, at least in part, key physiological functions (e.g., photosynthesis) and thereby seed and DM.

The present long-term continuous yield study enabled us to make an accurate assessment of the response of perennial fennel response to water-limited conditions. Owing to its natural habitat, this herbaceous medicinal species has evolved key osmoregulatory and antioxidative defense measures, making some of the fennel genotypes at least partially resilient to water limitation. The smallest water deficiency-induced reductions (ranging from 20 to 35%) in the seed yield of the 5th year were found in the genotypes Yazd, Bushehr, Urmia, Kashan, and Shiraz. The least water deficiency-associated reductions for the 6th year (ranging from 46 to 69%) were observed in Shiraz, Bushehr, Yazd, Avicenna, and Kashan. Interestingly, the greatest amount of the key osmoregulatory substance (i.e., proline) was detected in the inadequately watered plants (measured only in the 6th year) of

the genotypes Kashan, Isfahan, Shiraz, Avicenna, and Yazd. Hence, in contrary to a view arguing that osmotic adjustment mainly serves plant survival rather than productivity, fennel typifies a plant species encompassing certain genotypes with osmoregulatory measures beneficial to seed and dry mass production in water-limited circumstances. The presented data indicates for the first time that ratooning of fennel may lend us a hand in safeguarding sustainability and producing natural products with a high degree of environmental compatibility and additional economic advantage. Alike a majority of other perennial species (Angelina et al. 2009), not only ratooned fennel needs limited soil management practices, it also may be produced with minimal pesticide use, due to having few natural pests. These features bring about ecological advantages for ratooned fennel in comparison with conventional annual crop species.

4 Conclusions

Diversifying farming systems, sustaining biodiversity, and minimizing chemical and machinery inputs are especially crucial in areas where crop production practices have been extended into marginal conditions. Our interest in the potential role of fennel as a ratooned (and hence perennial) species had arisen from the suitability of such species for organic and sustainable production systems. Novelty of the present study lies in the finding that ratooning fennel may lead to the production of an acceptable seed and essential oil yield over a 6-year production cycle with minimum energy, labor, chemicals, and monetary inputs along with abating cultural practices. Based on the 6-year data gathered and osmoregulatory and antioxidative defense capabilities found, it can be concluded that certain fennel genotypes have the competence to maintain, at least in part, seeds/plant and hence seed yield and DM and seem reasonably suited for cultivation as a ratoon crop in erosion-threatened soils and drought-prone climatic conditions typically encountered in the Middle East countries such as Iran. Such novel findings are particularly valuable if put into action in arid and semi-arid regions anticipated to experience further environmental hardships due to the current climate change.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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