Industrial Crops & Products 128 (2019) 256–269

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Chemotypes and morpho-physiological characters affecting essential oil yield in Iranian cumin landraces

Moslem Bahmankar[a,*], Seyed Mohammad Mahdi Mortazavian[a,*], Masoud Tohidfar[b], Seyed Ahmad Sadat Noori[a], Ali Izadi Darbandi[a], Dhia Faleh Al-fekaiki[c]

[a] Department of Agronomy and Plant Breeding Sciences, College of Aburaihan, University of Tehran, Tehran, Iran
[b] Department of Plant Biotechnology, Faculty of Life Science and Biotechnology, Shahid Beheshti University, G.C. Tehran, Iran
[c] Department of Food Science, College of Agriculture, University of Basrah, Basrah, Iraq

ARTICLE INFO

Keywords:
Cuminal
GC-MS chromatography
Gene expression
Oil body
Photosynthetic pigments

ABSTRACT

The cumin essential oil is used in the food and pharmaceutical industry. Despite the large commercial production of cumin in Iran, there is no comprehensive study on quality and quantity of its essential oil. This experiment was conducted to study morpho-phenological and physiological characters affecting essential oil yield and to identify possible chemotypes in Iranian cumin landraces. Eyvanekey landrace with the highest essential oil content and yield had the highest carotenoids and total chlorophyll contents and oil body number in the cell. The results of the GC-MS chromatography showed that the identified components were classified into four organic compounds including monoterpenes, oxygenated monoterpenes, sesquiterpenes and others. According to essential oil compositions, cumin landraces were clustered in two chemotypes including low cuminal / high monoterpenes and high cuminal / low monoterpenes chemotypes, indicating a significant difference in essential oil quality. Khansar landrace with the highest cuminal content was introduced as the best Iranian landrace in terms of essential oil quality. Neronine alkaloid was identified in cumin essential oil for the first time, and Jat landrace is a novel plant source of neronine alkaloid. Essential oil yield had the positive regression and correlation coefficients with SWM (seed weight per m^2) and days to maturity which indicate that these characters are suitable selection indexes to improve cumin essential oil yield. A negative relationship between the cuminal content and limonene synthase gene expression (r = -0.55*) was observed. So, it was concluded that landraces having low limonene synthase gene expression are favorable for marketing.

1. Introduction

Cumin (*Cuminum cyminum* L.) belongs to Apiaceae family and the essential oil of cumin is used in the food and pharmaceutical industry (Derakhshan et al., 2010; Dubey et al., 2017). From ancient times, medicinal and spicy plants have been used as food condiments and also for the treatment of disease due to low toxic and the lack of side effects (Li et al., 2009). Cumin seeds are used in food, cosmetics, and perfume industries (Li and Jiang, 2004; Dubey et al., 2017). Cumin has medicinal properties for therapy of various diseases, especially digestive disorders, toothache, wound, and also it is known due to diuretic, antitumor, anti-inflammatory, antidiabetic, antifungal, antibacterial, antioxidant, and antispasmodic properties (Hemeda and Klein, 2006; Hajlaoui et al., 2010; Moghaddam et al., 2015). Aerial parts of cumin contain carotenoids, flavonoids, and anthocyanin; therefore they could be utilized as natural antioxidants (Panico et al., 2005). It is noteworthy that the highest contents of cumin bioactive compounds exist in reproductive organs especially seeds (Rebey et al., 2012).

Genetic variation plays an important role in plant breeding because hybrids obtained from the lines with more genetic variation show more heterosis compared to convergent races (Bahmankar et al., 2016). Variation is prerequisite for plant breeding and crops have been improved by selection of desirable genotypes during the former decades (Bahraminejad and Mohammadinejad, 2013). The knowledge of morpho-phenological and phytochemical diversity can lead to successful breeding programs in every region (Dubey et al., 2017; Moghaddam and Pirbalouti, 2017). Moghadam and Pirbaloti (2017) reported that cumin landraces were located in three groups which have significant differences in terms of all characters except the number of sub-umbels per umbel, and harvest index. The results of another study showed that there were significant differences between cumin landraces for all the measured agronomical characters (Bahraminejad et al.,

* Corresponding authors.
E-mail addresses: Bahmankar_64@ut.ac.ir (M. Bahmankar), Mortazavian@ut.ac.ir (S.M.M. Mortazavian).

https://doi.org/10.1016/j.indcrop.2018.10.080
Received 17 July 2018; Received in revised form 26 October 2018; Accepted 28 October 2018

2011).

Studies on the correlation between characters are important for plant breeding programs (Ali et al., 2003). However, the relationship between two variables may be affected by the third variable and correlation coefficient is not able to identify the factors causing relationships between variables (Bahmankar et al., 2014). Path analysis is a method to decompose correlation coefficients into direct and indirect effects and can provide useful information regarding the way in which characters influence each other. The direct selection of essential oil yield as a polygenic character is often not effective for improving it, while indirect selection of yield components is an effective breeding method (Karimzadeh-Asl et al., 2015). Path analysis was used frequently in medicinal plants (Lal, 2007; Bardideh et al., 2013; Karimzadeh-Asl et al., 2015). It was reported that the number of umbel per plant, and the number of seed per umbel had the most direct influence on cumin seed yield (Bahraminejad et al., 2011).

The major components of cumin essential oil were cumin aldehyde (cuminal), gamma-terpinene, beta-pinene, p-cymene, and safranal in different percentages (Jirovetz et al., 2005; Hajlaoui et al., 2010; Rebey et al., 2012; Moghaddam et al., 2015; Bahmankar et al., 2017; Dubey et al., 2017). Xie et al. (2011) reported that the compositions of cumin essential oil varied in different regions. There is a relationship between the concentration of essential oil compositions, and climatic and geographical situation (Li and Jiang, 2004). Gamma-terpinene/1-phenyl-1, 2-ethanediol, and cumin aldehyde/gamma terpinene chemotypes were reported for Tunisian and Indian cumin landraces, respectively (Bettaieb et al., 2011). Gamma-terpinene chemotype was also described for Spanish cumin landraces (Viuda-Martos et al., 2007). In another study, cumin aldehyde chemotype was reported (Beis et al., 2000). The cumin chemotypes are origin-dependent and different cumin chemotypes exist in a country (Moghaddam and Pirbalouti, 2017). Previous studies have suggested that endogenous factors including biochemical and physiochemical properties of the plant, as well as exogenous factors such as various environmental conditions, can form landraces and chemotypes in species (Barra, 2009; Moghaddam et al., 2015).

It is proved that limonene synthase gene has an important role at the beginning of biosynthesis pathway of terpenoids derivative compounds (Munoz-Bertomeu et al., 2008; Zarinkamar et al., 2012). On the other hand, it is well known that terpenoids are the major components of most plants essential oils and limonene is a precursor for monoterpenes and their derivatives (Zarinkamar et al., 2012). Limonene synthase gene expression positively affects the plant resistance to pests and diseases (Lucker et al., 2004, Zarinkamar et al., 2012).

Plant seeds store a high content of storage lipids which are applied as sources of carbon and energy for seed germination and seedling growth (Shimada and Hara-Nishimura, 2010). The storage lipids are present in small separate organelles that are called oil body. The diameters of oil bodies are variable and they range between 0.5 and 20 μm in different species and genotypes (Siloto et al., 2006).

Cumin is mainly cultivated in Iran, India, Morocco, Turkey, Syria, Greece, Egypt, Algeria, and China (Derakhshan et al., 2010; Dubey et al., 2017). It was noteworthy that Iran, Indian, Syria, Pakistan, USA, and Turkey are the main cumin exporter, and Iran provides 20-40% of the world production and export. Despite the large commercial production of cumin in Iran, no large study has been done on Iranian cumin landraces in terms of morpho-phenological and physiological characters, quality and quantity of essential oil and limonene synthase gene expression. The studied landraces in the current study are the main landraces distributed in many parts of Iran, and therefore, knowledge of essential oil yield and its composition and limonene synthase gene expression in these landraces are important for natural products research in the near future. The objectives of the current study were to: (1) assess the morpho-phenological variation in Iranian cumin landraces, (2) determine the correlation, multiple regression, and path analysis between essential oil yield and yield components and morpho-phenological characters and introduce the best selection index for the future cumin breeding programs, (3) evaluate the relationship between essential oil yield and photosynthetic pigments (4) assess the phytochemical variation in Iranian cumin landraces and try to identify its possible chemotypes, (5) introduce the best landrace in term of essential oil quality, and (6) evaluate the relationship of limonene synthase gene expression with monoterpenes and cuminal contents in essential oil of the selected cumin landraces.

2. Materials and methods

The current study was divided into four experimental sets including assessment of morpho-phenological and phytochemical variation in 20 Iranian cumin landraces, evaluation of photosynthetic pigments contents in five selected cumin landraces with high, medium, and low essential oil yield, measurement of oil body number in a cell of two selected landraces including Eyvanekey (with high essential oil content and yield) and Khatam (with low essential oil content and yield), and analysis of limonene synthase gene expression in five selected landraces with different contents of monoterpenes.

2.1. Plant materials

Seeds of 20 Iranian cumin landraces were supplied by seed bank of Aburaihan College, Tehran University (Figs. 1,2 , Table 1). This seed bank was established by the second author of the current study in 2011 by collecting landraces of main producer provinces from Iran. To eliminate the environmental effects, plants were propagated under the same conditions and their seeds were gathered and planted at the research farm of Abouraihan College, University of Tehran in 2016 and 2017. The research farm located in Pakdasht city (35° 28′ 54″ N, 51° 40′ 49″ E). Mean annual precipitation and mean annual temperature were 175 mm and 16.9 °C, respectively in 2016 and 173 mm and 16 °C, respectively, in 2017. This field was not under culture of any plant for one year. Each landrace was planted in 1 m^2 plots in a sandy–clay soil with pH 7.2. The soil was well drained. The cultural operations consisted manual elimination of weeds and normal irrigation in order to maintain soil moisture. Final plant density was 120 plants per m^2 as an optimum density. During the growing season no disease or pests were observed. Morpho-phenological characters, photosynthetic pigments contents, essential oil content and essential oil yield were measured in two years (2016 and 2017). Other characters (GC–MS analysis, oil body number in the cell and gene expression*)* were measured in 2017.

2.2. Morpho-phenological characters

Morpho-phenological characters including days to maturity, plant height (cm), plant mechanized height (cm) (mechanized height is the height from the ground to the first branch of the stem, which is important for the mechanized harvesting), umbellet per plant, seed per umbellet, single plant weight (g), biological weight per plot (g), seed weight per plot (g), and essential oil yield (kg ha^{-1}) were measured. These characters were recorded during two years (2016 and 2017). Each replication was the average of 50 sampled plants.

2.3. Essential oil yield and GC–MS analysis

The essential oil of seeds was extracted from dried powdered cumin seeds in Clevenger apparatus employing the procedure described by Bahmankar et al. (2018). The essential oil content was determined based on dry weight and expressed as percent (%w/w) and then essential oil yield (kg ha^{-1}) was calculated. Essential oil compositions were assessed by GC–MS (a combined analytical method to identify different substances of sample); Varian CP-3800 GC (Gas Chromatography) coupled with Varian 4000 (Ion trap) MS (Mass Spectrometry) equipped with a capillary VF-5 fused silica column (30m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at the

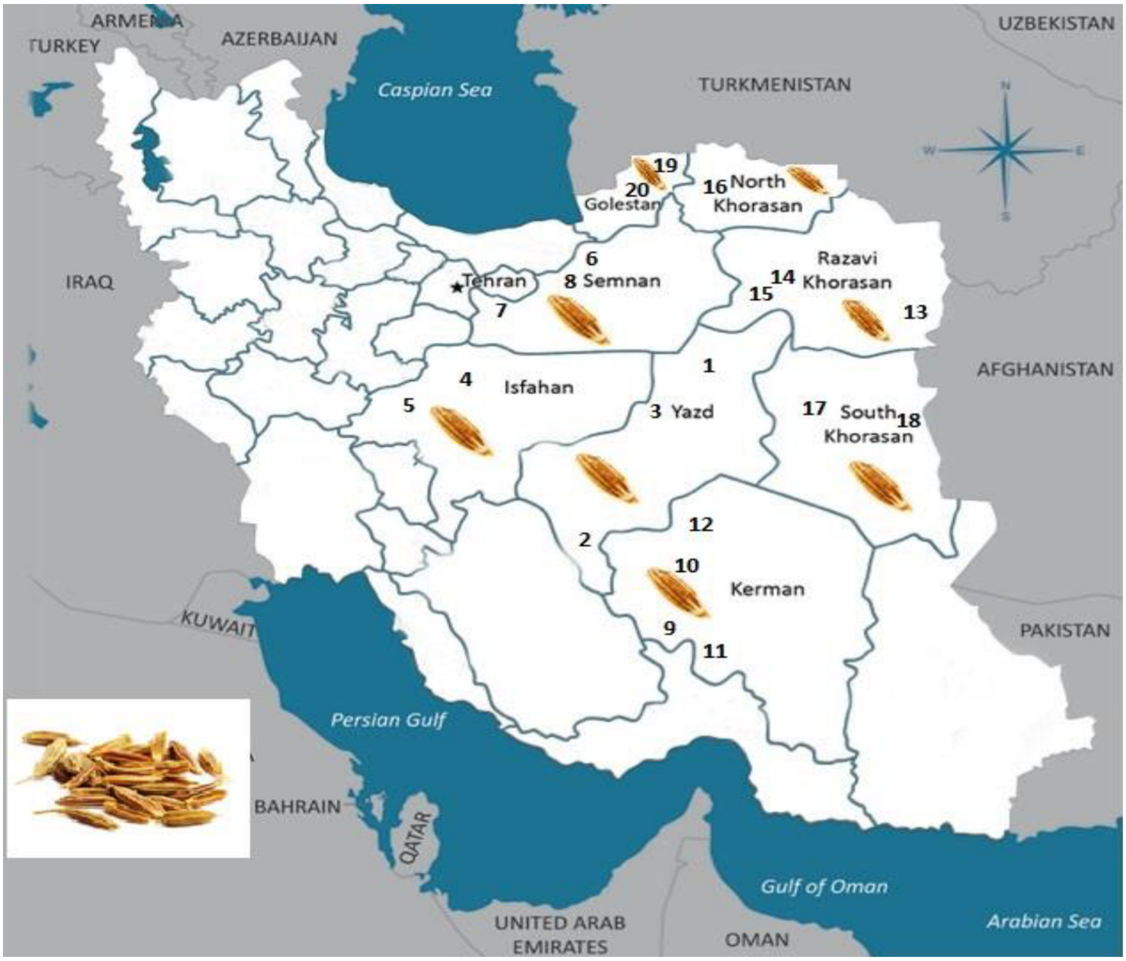

Fig. 1. Origins of 20 studied cumin landraces in map.

constant flow of 1.0 ml min^{-1}; split ratio, 1/50. Mass spectra were taken at 70 Ev and the Mass range was from m/z 35–400 a.m.u. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 3 °C min^{-1}, and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. The essential oil compositions were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6–C24) and the oil on a VF-5 column under the same chromatographic conditions. The compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature. For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors. Three replications of essential oils were analyzed to identify the chemical composition of each landrace. Only compounds that make up a significant proportion of the essential oil (≥ 0.04%) were shown in the current study.

2.4. Photosynthetic pigments contents

Khatam and Natanz landraces (with low essential oil yield, 1.00 and 2.50 kg ha^{-1}, respectively, Table 2), Darmiyan and Kuhbanan landraces (with medium essential oil yield, 6.90 and 6.60 kg ha^{-1}, respectively, Table 2), and Eyvanekey landrace (with high essential oil yield, 14.33 kg ha^{-1}, Table 2) were assessed in terms of photosynthetic pigments contents. Chlorophyll (Chl) a, Chl b, and carotenoids were measured employing the procedure described by Lichtenthaler and Buschman (2001). Each replication was the average of three sampled plants.

2.5. Oil body number in the cell

For observation of oil body, 10 seeds of two selected landraces including Eyvanekey [with high essential oil content (2.14%, Fig. 3) and yield (14.33 kg ha^{-1}, Table 2)] and Khatam [with low essential oil content (0.68, Fig. 3) and yield (1.00 kg ha^{-1}, Table 2)] were soaked in the distilled water for 6 hours and then their outer shell was removed. Cross-sections of the seeds were made with a Reichert sliding microtome and by hand–cutting and then were fixed in FAA. Sections were cleared in sodium hypochlorite and stained by carmine-vest (1% w/v in 50% ethanol) and methyl blue (1% w/v, aqueous). They were mounted in gelatin and observed by an optical microscope (Nikon YS 100). The number of oil body in the cell was counted.

2.6. Limonene synthase gene expression

Khatam landrace (with low monoterpenes content), Saduq and Maneh landraces (with medium monoterpenes contents), Ardekan and Eyvanekey landraces (with high monoterpenes contents) were selected to evaluate limonene synthase gene expression. Three replications were analyzed and three sampled plants were mixed together for each replication. Total RNA extraction, genomic DNA removal, cDNA synthesis, and qPCR were performed; employing the procedure described by Bahmankar et al. (2018). Amplicon efficiencies of two primer pairs were calculated with cDNA serial dilutions using this formula: $E = 10^{-1/slope}-1$. Relative expression amount was computed, using the $(1 + E)^{-\Delta\Delta CT}$ method (Pfaffi, 2001).

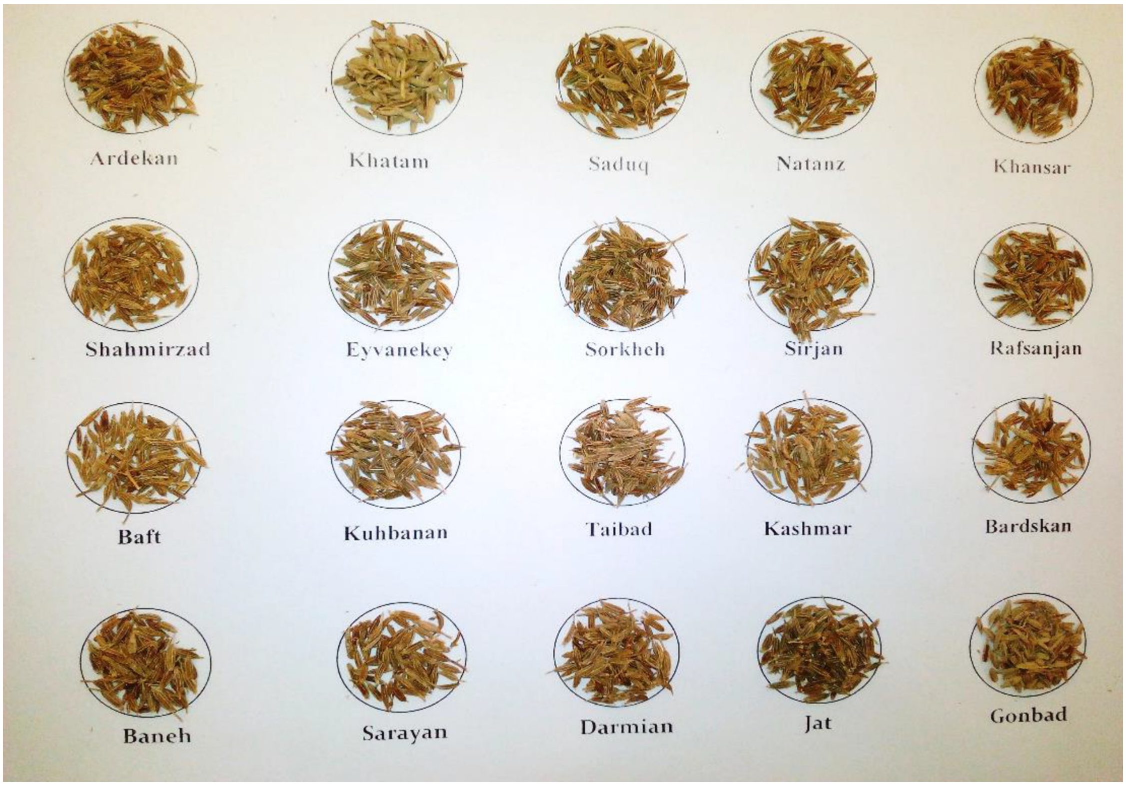

Fig. 2. Seeds of the 20 studied cumin landraces.

2.7. Statistical analysis

The data were first examined for the normality and were then analyzed based on a randomized complete block design with three replications. The Morpho-phenological characters, photosynthetic pigments contents, essential oil content and essential oil yield were measured in two years (2016 and 2017). The non-significant effects of genotype × year interactions were demonstrated using combined analysis of variance and therefore the average data of two years were applied for analysis. The experiment of GC-MS analysis, oil body number in the cell and limonene synthase gene expression was performed as a randomized complete block design with three replications in 2017. Mean comparisons were carried out, using Duncan's multiple range test and the results are expressed as the mean ± SE (standard error of the mean). The correlation coefficients were calculated between characters. In the multiple stepwise regressions, essential oil yield and morpho-phenological characters were considered as a dependent, and independent variables, respectively and then path analysis was done. The cluster analyses of morpho-phenological and phytochemical characters were done. Euclidean distance and wards method were used for cluster analysis. The SAS 9.2, SPSS 16, Minitab 16, and Excel software packages were used to analyze the data, and draw the graphs.

Table 1
Local information of 20 studied Iranian cumin landraces.

Landrace code	Landraces	Province	Altitude(m)	Latitude	Longitude	Annual mean precipitation (mm)
1	Ardekan	Yazd	1035	32°19′ N	54° 1′ E	58
2	Khatam	Yazd	1600	31°30′ N	54° 40′ E	300
3	Saduq	Yazd	1175	32° 33′ N	54° 12′ E	68
4	Natanz	Isfahan	1710	33° 30′ N	51° 55′ E	97
5	Khansar	Isfahan	2078	33° 13′ N	50° 18′ E	198
6	Shahmirzad	Semnan	2004	35° 17′ N	53° 32′ E	129
7	Eyvanekey	Semnan	1077	35° 34′ N	52° 06′ E	127
8	Sorkheh	Semnan	1149	35° 46′ N	53° 21′ E	129
9	Sirjan	Kerman	1740	29° 45′ N	55° 17′ E	168
10	Rafsanjan	Kerman	1545	30° 35′ N	56° 00′ E	126
11	Baft	Kerman	2266	29° 23′ N	56° 00′ E	204
12	Kuhbanan	Kerman	1945	31° 25′ N	56° 17′ E	146
13	Taibad	Razavi Khorasan	180	33° 44′ N	60° 46′ E	240
14	Kashmar	Razavi Khorasan	1057	35° 24′ N	58° 46′ E	178
15	Bardskan	Razavi Khorasan	987	35° 26′ N	57° 17′ E	154
16	Maneh	North Khorasan	850	37° 16′ N	57° 19′ E	210
17	Sarayan	South Khorasan	1436	33° 58′ N	58° 05′ E	145
18	Darmiyan	South Khorasan	1373	32° 32′ N	59° 28′ E	150
19	Jat	Golestan	194	37° 38′ N	55° 42′ E	315
20	Gonbad	Golestan	40	36° 24′ N	55° 18′ E	363

Table 2
Means comparison of measured morpho-phenological characters in the studied cumin landraces.

Landraces	PH	MH	UP	SU	SPW	BWM	SWM	SW	EO Yield (kg ha^{-1})	DM
Ardekan	20.60^{e-h}	13.27^{a}	28.17^{bc}	5.87^{a-d}	2.31d^{ef}	149.00^{fg}	53.87^{h}	4.67^{abc}	10.45^{c}	102.67^{n}
Khatam	19.73^{h}	11.67^{b-f}	21.73^{h}	6.00^{a-d}	2.49^{b-f}	91.33^{h}	14.67^{k}	3.33^{h}	1.00^{n}	105.00^{m}
Saduq	22.07^{a-f}	11.73^{b-f}	22.53^{gh}	6.07^{abc}	1.81^{g}	273.00^{a}	100.67^{a}	4.67^{abc}	10.57^{bc}	110.33^{hij}
Natanz	22.00^{a-g}	10.93^{ef}	22.33^{gh}	6.33^{a}	2.25^{d-g}	108.00^{h}	32.27^{ij}	3.87^{d-h}	2.50^{m}	111.00^{ghi}
Khansar	21.51^{b-h}	10.93^{ef}	24.58^{fg}	5.34^{b-f}	2.17^{efg}	151.67^{f}	67.00^{efg}	3.67^{fgh}	10.20^{d}	117.67^{c}
Shahmirzad	19.93^{gh}	12.00^{a-f}	21.67^{h}	6.20^{ab}	2.03^{fg}	129.67^{g}	39.13^{i}	3.67^{fgh}	3.20^{l}	108.00^{kl}
Eyvanekey	23.13^{a-d}	13.13^{a}	27.27^{cde}	6.53^{a}	2.65^{b-e}	193.33^{cd}	67.00^{efg}	4.93^{ab}	14.33^{a}	121.00^{a}
9Sorkheh	21.67^{b-g}	11.00d^{ef}	27.00^{c-f}	4.67^{f}	2.22^{d-g}	167.67^{ef}	69.33^{ef}	3.78^{e-h}	8.08^{h}	118.00^{bc}
Sirjan	23.33^{abc}	12.10^{a-e}	25.33^{def}	5.29^{c-f}	2.81^{abc}	185.00^{cde}	77.67^{cd}	3.45^{gh}	10.62^{b}	112.00^{efg}
Rafsanjan	22.40^{a-f}	12.87^{ab}	30.13^{ab}	6.40^{a}	2.83^{abc}	177.00^{de}	87.00^{b}	4.40^{bcd}	10.73^{b}	114.33^{d}
Baft	20.32^{fgh}	11.35^{c-f}	24.90^{ef}	5.39^{b-f}	2.85^{abc}	103.00^{h}	30.07^{j}	4.20^{c-f}	5.67^{i}	112.67^{ef}
Kuhbanan	23.33^{abc}	12.33^{a-d}	31.00^{a}	5.67^{b-e}	2.27^{d-g}	193.33^{cd}	82.00^{bcd}	4.33^{b-e}	6.60^{i}	107.33^{l}
Taibad	22.17^{a-f}	11.50^{b-f}	25.00^{ef}	5.33^{b-f}	2.38^{c-f}	184.00^{cde}	82.33^{bcd}	4.37^{b-e}	9.00^{g}	109.67^{ij}
Kashmar	21.00^{d-g}	10.67^{f}	25.33^{def}	5.17^{def}	2.48^{b-f}	172.33^{ed}	70.00^{ef}	3.96^{d-g}	10.97^{b}	119.33^{b}
Bardskan	23.54^{ab}	11.48^{b-f}	30.39^{ab}	4.9^{4ef}	2.43^{c-f}	177.33^{de}	59.17^{gh}	4.87^{ab}	3.77^{k}	109.33^{jk}
Maneh	22.67^{a-e}	12.40^{abc}	28.87^{abc}	6.53^{a}	2.59^{b-e}	194.33^{cd}	85.67^{bc}	4.77^{abc}	10.48^{c}	118.00^{bc}
Sarayan	22.00^{a-g}	13.33^{a}	29.00^{abc}	4.67^{f}	2.67^{a-d}	188.33^{cde}	80.00^{bcd}	3.63^{fgh}	9.47^{f}	117.33^{c}
Darmiyan	21.33^{c-h}	12.67^{abc}	27.60^{cd}	6.20^{ab}	2.93^{ab}	176.33^{de}	61.00^{gh}	4.20^{c-f}	6.90^{i}	111.67^{fgh}
Jat	22.27^{a-f}	12.07^{a-e}	29.20^{abc}	6.13^{abc}	3.13^{a}	224.67^{b}	65.40^{fg}	4.33^{b-e}	9.04^{g}	113.33^{de}
Gonbad	24.05^{a}	12.70^{abc}	27.19^{cde}	5.40^{b-f}	2.05^{gf}	200.67^{c}	74.67^{de}	5.00^{a}	9.84^{e}	117.33^{c}

In each character, landraces with one common letter at least are not significantly difference at the 5% level.
PH: Plant Height (cm), MH: Mechanized Height (cm), UP: Umbellet per Plant, SU: Seed per Umbellet, SPW: Single Plant Weight (g), BWM: Biological Weight per m^2 (g), SWM: Seed Weight per m^2 (g), SW: 1000-seed weight (g), EO: Essential Oil Yield (kg ha^{-1}), DM: Days to Maturity.

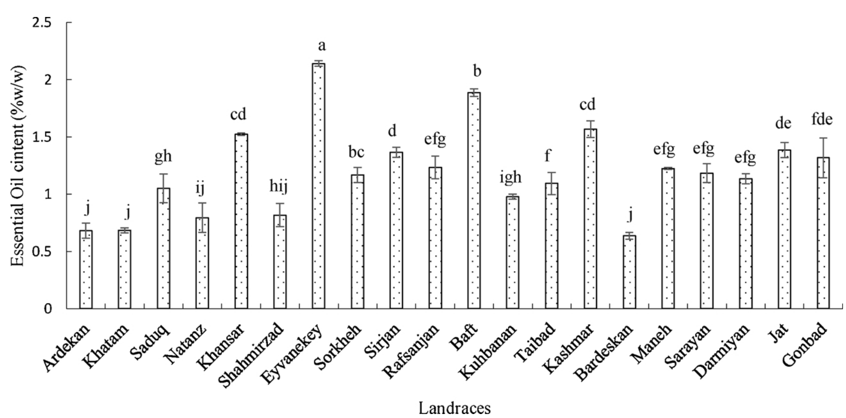

Fig. 3. Essential oil of dried seeds in studied cumin landraces.

3. Results

3.1. Morpho-phenological characters

Results of variance showed the strong influence of landrace on the most measured morpho-phenological characters (data not shown), which indicated the high morpho-phenological variation among them. The Mean of essential oil yield was 8.17 kg ha^{-1} and ranged from 14.33 kg ha^{-1} for Eyvanekey landrace to 1.00 kg ha^{-1} for Khatam Landrace (Table 2). The mean of days to maturity was 112.80 d and ranged from 102.67 d (Ardekan landrace) to 121.00 d (Eyvanekey landrace, Table 2). The mean of biological weight per m^2 (BWM) was 172.00 g and ranged from 91.33 g (Khatam landrace) to 224.67 g (Jat landrace, Table 2). The mean of seed weight per m^2 (SWM) was 64.94 g and ranged from 14.67 g (Khatam landrace) to 100.67 g (Saduq landrace, Table 2).

The mean comparison of phenological characters suggested that the studied cumin landraces were clustered in three groups including early maturity [Ardekan (102.67 d) and Khatam (105.00 d)], medium maturity [Sirjan (112.00 d), Kuhbanan (107.33 d), Taibad (109.67 d), Natanz (111.00 d), Bardskan (109.33 d), Darmiyan (111.67 d), Jat (113.33 d), Saduq (110.33 d), Baft (112.67 d), Rafsanjan (114.33 d), Shahmirzad (108.00 d)], and late maturity [Gonbad (117.33 d), Sorkheh (118.00 d), Kashmar (119.33 d), Sarayan (117.33 d), Khansar (117.67 d), Eyvanekey (121.00 d), Maneh (118.00 d)]. The highest essential oil content and yield were obtained in the latest maturing landrace (Eyvanekey landrace with the mean of 2.14% and 14.33 kg ha^{-1} for essential oil content and essential oil yield, respectively; Table 2).

Essential oil yield had positive and significant correlations with BWM ($r = 0.64^{**}$), SWM (0.74^{**}), and days to maturity ($r = 0.58^{**}$). A high correlation coefficient was found between BWM and SWM (0.88^{**}, Table 3).

In the multiple stepwise regressions, essential oil yield, and all other morpho-phenological characters (Table 2) were considered as dependent, and independent variables, respectively and the results showed that 64% of total variation in essential oil yield could be explained by two characters including SWM (54%) and days to maturity (10%, Table 4). The regression coefficients of two characters entered into regression model were significant (Table 4).

Seed weight mass had the most direct effect (0.60^{**}) on essential oil yield (Table 5). The indirect effect of SWM was also positive (0.14) via days to maturity (Table 5). The days to maturity had positive and significant direct effect (0.35^{*}) on essential oil yield and its indirect effect (0.23) was positive through SWM (Table 5).

Four morpho-phenological clusters were obtained by cluster analysis (Ward method and Euclidean distance, Fig. 4). The second group (C2) included Darmiyan (South Khorasan), Jat (Golestan), Eyvanekey (Semnan), Rafsanjan (Kerman), and Maneh (North Khorasan, Fig. 4).

Table 3
The correlation coefficient among 13 studied characters in 20 cu min landraces.

Characters	PH	MH	UP	SU	SPW	BWM	SWM	SW	EO Yield	DM	Altitude	Latitude	Longitude
PH	1												
MH	0.26 ^{ns}	1											
UP	0.56**	0.31^{ns}	1										
SU	-0.09^{ns}	0.45*	-0.13^{ns}	1									
SPW	0.02^{ns}	0.17^{ns}	0.42^{ns}	0.12^{ns}	1								
BWM	0.62**	0.08^{ns}	0.40^{ns}	0.01^{ns}	-0.06^{ns}	1							
SWM	0.63**	0.06^{ns}	0.47*	-0.11^{ns}	-0.09^{ns}	0.88**	1						
SW	0.52**	0.28^{ns}	0.49*	0.29^{ns}	-0.06^{ns}	0.51*	0.40^{ns}	1					
EO Yield	0.41^{ns}	0.15^{ns}	0.36^{ns}	0.01^{ns}	0.13^{ns}	0.64**	0.74**	0.41^{ns}	1				
DM	0.35^{ns}	-0.10^{ns}	0.19^{ns}	-0.15^{ns}	0.17^{ns}	0.29^{ns}	0.39^{ns}	0.12^{ns}	0.58**	1			
Altitude	-0.42^{ns}	-0.08^{ns}	-0.29^{ns}	0.02^{ns}	-0.02^{ns}	-0.53*	-0.37^{ns}	-0.57**	-0.36^{ns}	-0.18^{ns}	1		
Latitude	0.24^{ns}	-0.04	0.18^{ns}	0.02^{ns}	-0.14^{ns}	0.33^{ns}	0.16^{ns}	0.36^{ns}	0.16^{ns}	0.44*	-0.65**	1	
Longitude	0.1^{ns}	-0.06^{ns}	0.36^{ns}	-0.22^{ns}	0.39^{ns}	0.23^{ns}	0.29^{ns}	0.16^{ns}	0.03^{ns}	-0.05^{ns}	-0.38^{ns}	0.01^{ns}	1

ns,*, and **: not significant, significant at $P \leq 0.05$, and significant at $P \leq 0.01$, respectively.
PH: Plant Height, MH: Mechanized Height, UP: Umbellet per Plant, SU: Seed per Umbellet, SPW: Single Plant Weight (g), BWM: Biological Weight per m^2 (g), SWM: Seed Weight per m^2 (g), SW: 1000-seed weight (g), EO: Essential Oil Yield (kg ha^{-1}), DM: Days to Maturity.

The third group (C3) included Sorkheh (Semnan), Kashmar (Razavi Khorasan), and Khansar (Isfahan, Fig. 4) landraces. The landraces located in the second and third groups were late maturity (115. 67 d and 118.33 d, respectively, Table 2) and they had a high essential oil yield (10.30 and 9.75 kg ha^{-1}, respectively, Table 2). Khatam (Yazd), Shahmirzad (Semnan), Natanz (Isfahan), and Baft (Kerman) landraces were located in the fourth group (C4, Fig. 4). The landraces of the fourth group were early maturity (109.17 d, Table 2) with low essential oil yield (3.09 kg ha^{-1}, Table 2). The landraces located in the same group belong to different provinces of Iran, with different weather conditions (Fig. 4, Table 1 Table 6)

3.2. Phytochemical compounds

The results of the GC-MS chromatography showed that the identified components were classified into four groups of organic compounds including monoterpenes, oxygenated monoterpenes, sesquiterpenes and others (Table 7). Monoterpenes (20.96%) and oxygenated monoterpenes (62.86%) were the major compounds in the cumin essential oils and 47% of oxygenated monoterpenes were related to cuminal (Table 7). The number of identified essential oil compounds in landraces was ranged from 25 (in the Natanz landrace) to 50 (in the Jat, Saduq, and Sorkheh landraces, data not shown). Cuminal content ranged from 16.53% in Saduq landrace to 42.30 % in Khansar landrace (Table 7). Jat (0%) and Ardekan (17.10%) landraces had the least and the most content of γ-Terpinene, respectively (Table 7). Ardekan and Sarayan landraces had the highest content of α-Cedrene (1.55%) and Carotol (1.28%), respectively (Table 7). In the current study, Neronine alkaloid with the chemical formula of $C_{18}H_{21}NO_6$ (Fig. 7) and molecular weight of 347 g/mol was observed only in the Jat landrace originated from Golestan province and it was about 2.02% of essential oil (Table 7).

The landraces were grouped in two chemotypes by cluster analysis which had the significant difference in essential oil quality (Ward's method and Euclidean distance, Fig. 5, Table 8). In the current study, Kuhbanan, Natanz, Bardskan, Khatam, Khansar, Baft, and Rafsanjan landraces were located in the second chemotype (Fig. 5). The phytochemical profile of this chemotype showed that the cuminal content in its essential oil was high (Fig. 5, Table 8). The second chemotype had fewer contents of monoterpenes (13.98%) and sesquiterpenes (0.23) compared to first chemotype (24.72% and 1.39%, respectively, Table 8). The landraces located in the second chemotype were originated from different climate conditions in Iran (Figs. 6, 7).

Table 5
Direct and indirect effects of remained characters in the regression model on essential oil yield in cumin landraces using path coefficients analysis.

Characters	Correlation	Direct effects	Indirect effects via Maturity 1000-seed weight
SWM	0.74**	0.60**	0.14
Maturity	0.58**	0.35*	0.23

Residual effects = 0.1., *and ** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

3.3. Photosynthetic pigments contents

Eyvanekey landrace with the highest essential oil yield (14.33 kg ha^{-1}, Table 2) possessed the highest carotenoids contents (72.80 mg g^{-1} FW) and Chl a + b (52.00 mg g^{-1} FW) and Khatam with least essential oil yield had the least carotenoids content (40.00 mg g^{-1} FW, Fig. 8). The least Chl a + b content was observed in Natanz (19.50 mg g^{-1}FW) and Khatam landraces (22.00 mg g^{-1} FW, Fig. 8). Essential oil yield possessed high correlation with carotenoids (r = 0.91**) and Chl a + b (r = 0.95**).

Table 4
The results of stepwise multiple regression of essential oil yield and nine morpho-phenological characters (PH, MH, UP, SU, SPW, BWM, SWM, SW, and DM) in cumin landraces.

Regression equations	Coefficient of partial determination	Coefficient of Cumulative determination	c(p)	pr ≥ f
Essential oil yield = 0.63 + 0.12 SWM	0.54	0.54	2.63	0.00**
Essential oil yield = -24.62 + 0.09 SWM + 0.24 Maturity	0.10	0.64	0.46	0.04*

**significant at $P \leq 0.01$.
PH: Plant Height (cm), MH: Mechanized Height (cm), UP: Umbellet per Plant, SU: Seed per Umbellet, SPW: Single Plant Weight (g), BWM: Biological Weight per m^2 (g), SWM: Seed Weight per m^2 (g), SW: 1000-seed weight (g), and DM: Days to Maturity.

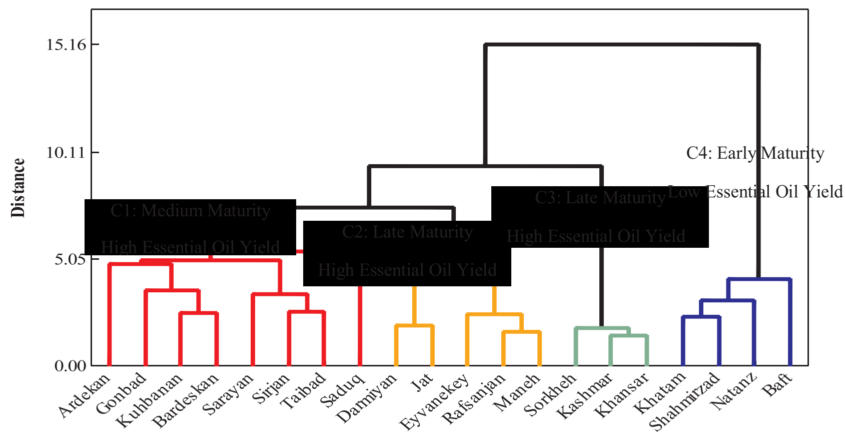

Fig. 4. Dendrogram of cluster analysis of the studied landraces using ward method based on morpho-phenological characters.

Table 6
Results of analysis of variance and mean comparison of traits for cluster analysis groups based on measured morpho-phenological characters.

H	MS between groups	MS within groups	Mean group			
			Group 1	Group 2	Group 3	Group 4
PH	4.67^{**}	0.88	22.64a	22.36a	21.39ab	20.50b
MH	2.10^{**}	0.40	12.30a	12.63a	10.86b	12.04a
UP	29.67^{**}	4.64	27.33a	28.61a	25.64ab	22.66b
SU	1.47^{**}	0.15	5.40b	6.36a	5.06b	5.98a
SPW	0.29^{*}	0.08	2.34b	2.82a	2.29b	2.41ab
BWM	7542.67^{**}	709.19	193.83a	193.13a	163.89a	108b
SWM	2191.74^{**}	149.78	76.30a	73.21a	68.78a	29.03b
SW	0.68^{*}	0.19	4.37ab	4.55a	3.80b	3.77b
EO Yield	45.44^{**}	5.39	8.79a	10.30a	9.75a	3.09b
DM	73.12^{*}	16.35	110.75bc	115.67ab	118.33a	109.17c

*, and **: significant at $P \le 0.05$ and $P \le 0.01$, respectively.
PH: Plant Height, MH: Mechanized Height, UP: Umbellet per Plant, SU: Seed per Umbellet, SPW: Single Plant Weight (g), BWM: Biological Weight per m^2 (g), SWM: Seed Weight per m^2 (g), SW: 1000-seed weight (g), EO: Essential Oil Yield (kg ha^{-1}), DM: Days to Maturity.

3.4. Oil body number in the cell

Two landraces including Eyvanekey [as late maturity (121 d, Table 2) landrace with high essential oil content and yield (2.14% and 14.33 kg ha^{-1}, respectively, Fig. 3, Table 2)] and Khatam (as early maturity landrace (105 d, Table 2) with low essential oil content and yield (0.68% and 1.00 kg ha^{-1}, respectively, Fig. 3, Table 2) were assessed in terms of oil body number in the cells. The results showed that the oil body number in a cell of Eyvanekey landrace (5.50 no. /cell) was significantly higher than that (1.90 no. /cell) in Khatam landrace (Fig. 9, 10).

3.5. Limonene synthase gene expression

Five cumin landraces including Khatam (with low monoterpenes content, 12.80%), Saduq and Maneh (with medium monoterpenes content, 23.63% and 24.60%, respectively), Ardekan and Eyvanekey (with high monoterpenes content, 34.20% and 33.95%, respectively, Table 7) were assessed. The tested landraces varied significantly ($P \le 0.01$) in terms of limonene synthase gene expression. Ardekan landrace had the highest expression of limonene synthase gene (19. 20 fold, Fig. 11) followed by Eyvanekey landrace (16.70 fold, Fig. 11). The least expression of limonene synthase gene (1 fold, Fig. 11) was observed in Khatam landrace. There was a significant and positive correlation ($r = 0.97^{**}$) between monoterpenes content and limonene synthase gene expression.

4. Discussion

4.1. Morpho-phenological characters and essential oil yield

There was high morpho-phenological variation between landraces. The high genetic variation can be used in the mass selection breeding program or developing new breeding population (Bahraminejad et al., 2011; Moghaddam and Pirbalouti, 2017). Cumin landraces were classified as early, medium, and late maturity. The most studied cumin landraces were late (35%) and medium maturity (55%), and only 10% of them were early maturity. Variation of maturity period in cumin landraces was reported by other studies (Bahmankar et al., 2016; Moghaddam and Pirbalouti, 2017). Early maturing landraces are very important for the regions with terminal stresses. The mean of 1000-seeds weight was 4.21 g and ranged from 3.33 g (Khatam landrace) to 5 g (Gonbad landrace, Table 2). The 1000-seeds weight in the current study was higher than the 1000-seeds weight of Iranian cumin landraces assessed by Moghadam and Pirbaloti (2017). The tested landraces varied significantly ($P \le 0.01$) for essential oil yield and morpho-phenological characters; hence, it is possible to select suitable landraces with different maturity period for cumin breeding program. Also, Bahraminejad et al. (2011) reported morphological variation in cumin landraces. Nevertheless it should be noted that the morphological characters are influenced by the environment, developmental, genetic factors, and their interaction (Labra et al., 2004).

The cumin essential oil is used in the food industry as an antimicrobial agent for preservative purposes (Hajlaoui et al., 2010; Xie et al., 2011; Mohammadpour et al., 2012). Essential oil content in studied cumin landraces ranged from 0.64% for Bardskan landrace to 1.89% for Baft landrace. It should be noted that the oil content of cumin in the current study was higher than that in study of Jalali-Heravi et al. (2007) on the Iranian cumin landraces and results of the current study were approximately similar to report of Hashemian et al. (2013). In the other studies, the essential oils content of Chinese cumin landraces (Li and Jiang 2004), Bulgarian (Jirovetz et al., 2005), Indian cumin landraces (Sowbhagya et al., 2008), and Tunisia (Rebey et al., 2012) were observed 3.80%, 5.30%, 2.30%, and 1.60%, respectively (Li and Jiang, 2004; Jirovetz et al., 2005; Sowbhagya et al., 2008; Rebey et al., 2012). Essential oil yield had positive and significant correlations with BWM ($r = 0.64^{**}$), SWM (0.74^{**}), and days to maturity ($r = 0.58^{**}$, Table 3). Safari et al. (2015) showed that seed weight is an important character in grain yield improvement. The positive relationship between essential oil yield and days to maturity may be due to most photosynthesis and growing period of late maturing landraces and therefore high photosynthetic rates result in increased essential oil production (Bardideh et al., 2013; Moghaddam et al., 2015). Other researchers have also verified the high capacity of essential oils biosynthesis in late-maturing genotypes of medicinal plants such as cumin and fennel (Bahmani et al.,

Table 7
Major chemical composition of the essential oil in 20 cumin landraces.

Compound **Monoterpenes**	[a]RI	Ardekan **34.2**	Gonbad **30.4**	Sorkheh **23.8**	Khashmar **30.5**	Sarayan **29.9**	Sirjan **30.6**	Kuhbanan **13.2**	Taibad **12.8**	Khatam **12.8**	Natanz **13.0**	Bardskan **15**	Khansar **10.84**	Darmiyan **16.62**	Eyvanekey	**16.7**	**23.63**	**15.27**	**17.77**	**13.71**	**24.6**
β- Pinene	943	9.95	9.4	1.96	9.34	8.18	7.57	2.58	2.41	2.23	2.55	2.84	1.77	3.32	8.82	7.99	6.14	4.67	4.85	2.53	7.5
p- Cymene	1042	4.99	5.42	2.53	10.67	5.85	5.84	3.39	3.11	5.14	3.37	3.41	4.51	3.22	6.33	6.71	2.81	4.31	3.85	4.5	5.5
γ-Terpinene	998	17.1	13.4	5.03	9.02	13.36	14.8	6.11	6.11	4.5	6.04	7.13	3.91	8.02	16.27	0	13.41	5.53	8.2	5.3	9.12
α-Thujene	1090	0.19	0.14	12.8	0.12	0	0.14	0.06	0	0	0.06	0.07	0	0.08	0.14	0.12	0.11	0.07	0.05	0.06	0.06
α-Phellandrene	969	0.72	0.78	0.51	0.46	1.21	1.05	0.45	0.58	0.56	0.41	0.91	0.16	0.87	0.84	0.55	0.4	0.21	0.2	0.55	0.85
α-Pinene	948	0.54	0.44	0.07	0.41	0.35	0.37	0.13	0.13	0	0.13	0.13	0	0.16	0.41	0.37	0.31	0.19	0.15	0.12	0.37
β-Phellandrene	964	0	0.5	0.14	0.46	0.39	0.45	0.21	0.19	0.15	0.2	0.22	0.14	0.27	0.51	0.46	0.37	0.29	0.25	0.2	0.21
Myrcene	958	0.55	0.19	0.54	0	0.61	0.27	0.25	0.24	0.25	0.28	0.2	0.35	0.6	0.53	0.44	0	0	0.22	0.45	0.55
-4-Carene	919	0.14	0.09	0.27	0	0	0.1	0.06	0	0	0	0.07	0	0.08	0.1	0.06	0.08	0	0	0	0.42
Oxygenated Monoterpenes		**55.44**	**64.17**	**38.2**	**62.56**	**60.22**	**64.62**	**84.37**	**70.47**	**80.08**	**84.9**	**82.97**	**85.35**	**54.16**	**61.72**	**60.4**	**36.48**	**78.92**	**79.98**	**66**	**50.15**
Nonene	1117	9.82	11.3	7.24	5.2	8.51	11.3	9.7	0.08	14.7	9.5	9.38	12.3	12.9	12.07	12.5	7.88	8.86	9.5	9.5	12
Silane	1115	0	0.56	1.11	9.14	0.98	0.4	0.18	0.19	1.88	0.45	0.19	2.72	0.11	0.9	1.09	0	5.03	4.95	1.5	0.9
Dimethoxytoluene	1080	0	0.28	0.97	13.67	0	0.24	0	0	1.24	0.54	0	0	0.13	0.79	0.98	0.06	4.94	4.5	1.2	0.75
Safranal	1270	0.05	0.05	0.11	0.29	0.12	0	0	0.28	0	0.09	0.16	0	0	0.33	1.37	0.21	0.21	0	0	0
Cuminal	1230	23.6	30.5	24.5	23.1	31.8	29.4	35.7	37.3	36.1	36.7	34.7	42.3	34.1	25.8	25.1	16.53	30.8	30.2	32.2	31
Carboxaldehyde	1175	3.87	4.38	4.27	1.66	2.27	3.58	6.15	3.24	3.66	7.02	7.25	3.25	6.68	5.57	3.56	2.96	2.68	5.23	3.6	5.5
Chavibetol	1318	0	0	0	0	0	0	0.23	0.18	0	0	0.25	1.48	0	0	0	0	0	0	0	0
Trans-Shisool	1250	0	0	0	0	0	0	0.11	0	0	0	0.14	0	0.18	0	0	0	0	0	0	0
Phenyl glycol	1453	18.1	17.1	0	9.5	16.54	19.7	32.3	29.2	22.5	30.6	30.9	23.3	0.06	16.26	15.8	8.84	26.4	25.6	18	0
Sesquiterpenes		**4.08**	**1.78**	**0.38**	**1.6**	**3.12**	**1.07**	**0.14**	**0.36**	**0.35**	**0.12**	**0.15**	**0.49**	**0.35**	**1.34**	**1.46**	**1.07**	**0.23**	**0.11**	**0.27**	**1.24**
α-Cedrene	1403	1.55	0.62	0.24	0.68	1.22	0.67	0.07	0.2	0.16	0.06	0.08	0.28	0.27	0.57	0.7	0.34	0.16	0.06	0.07	0.6
Dodecatrien	1564	1.32	0.58	0.04	0.38	0.62	0	0	0	0	0	0	0	0	0.35	0.4	0.34	0	0	0	0.32
Carotol	1593	1.01	0.33	0.1	0.31	1.28	0.4	0.07	0.16	0.19	0.06	0.07	0.21	0.08	0.3	0.26	0.27	0.07	0.05	0.2	0.21
Caryophyllene	1494	0.2	0.25	0	0.23	0	0	0	0	0	0	0	0	0	0.12	0.1	0.12	0	0	0	0.11
Others		0	0.43	0.32	0.12	0.79	0.59	0.35	0.25	0.48	0.34	0.28	0.29	0.31	0.46	2.65	0.21	0.35	0.32	0.32	0.45
Nonanal	1104	0	0.43	0.32	0.12	0.79	0.59	0.35	0.25	0.48	0.34	0.28	0.29	0.31	0.46	0.63	0.21	0.35	0.32	0.32	0.45
Neronine	2747	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.02	0	0	0	0	0

Compounds that make up less than 0.04% of essential oil are not reported.
[a] RI: retention index.

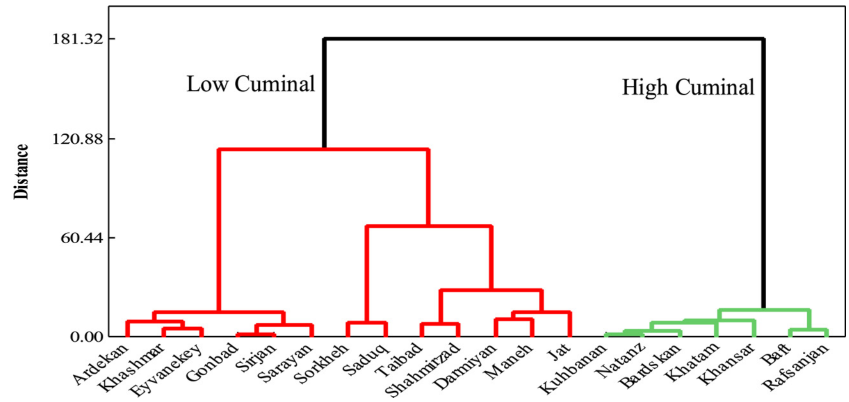

Fig. 5. Dendrogram of cluster analysis of studied landraces using ward method based on essential oil components.

Table 8
Results of analysis of variance and mean comparison of traits for cluster analysis groups based on chemical composition of the essential oil.

Traits	MS between groups	MS within groups	Mean group	
			Group 1	Group 2
Cuminal	232.14**	26.20	28.07b	35.21a
Terpenes	790.79*	113.81	83.39b	96.58a
Monoterpenes Hydrocarbons	524.93**	40.41	24.72a	13.98b
Oxygenated Monoterpenes	2864.49**	73.36	57.28b	82.37a
Sesquiterpene Hydrocarbons	6.19*	0.84	1.39a	0.23b

* and **: significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

2015; Moghaddam and Pirbalouti, 2017). Altitude possessed a negative correlation with BWM (r = -0.53*) and 1000-seed weight (r= -0.57**, Table 3). Bahmankar et al. (2018) reported the significant and negative correlations between altitude and photosynthetic pigments. As the altitude increases, humidity and temperature are reduced and resulted in a reduction of the photosynthetic pigments (Bahmankar et al., 2018). So it is concluded that variation of morpho-physiological traits in cumin are related to variation in geographical and weather situation of their origins.

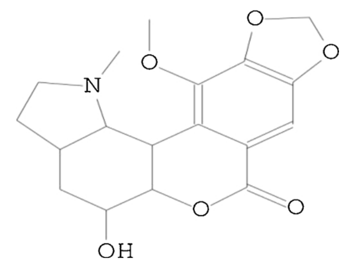

Fig. 7. Chemical structure of Neronine in the Jat landrace.

Multiple stepwise regression indicated that 64% of total variation in essential oil yield could be explained by two characters including SWM (54%) and days to maturity (10%, Table 4). The regression could not justify 36% of the variation. The other components may be able to explain 36% of the variation. It was reported that the number of seeds per plant accompanied with the number of umbrellas per plant, 1000-seed weight, and seed number in umbrella justified 89% of total variation in plant yield (Bahraminejad et al., 2011). Regarding regression, the direct and indirect effects of characters on essential oil yield were

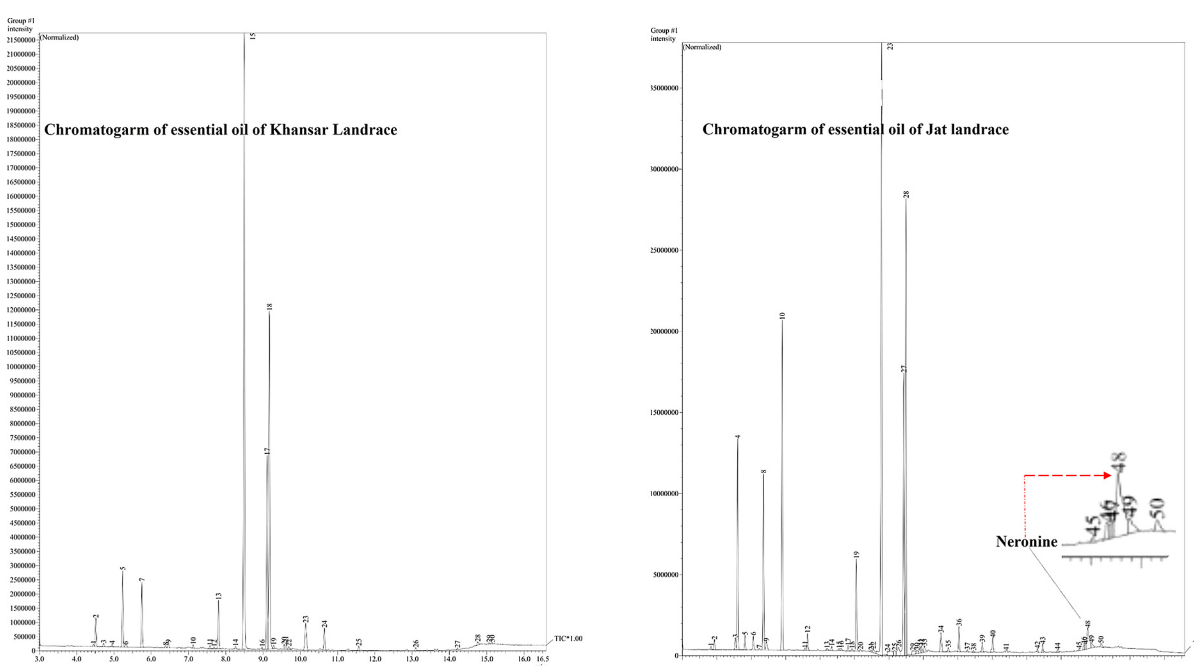

Fig. 6. Essential oil chromatograms of Khansar (a) and Jat (b) cumin landraces.

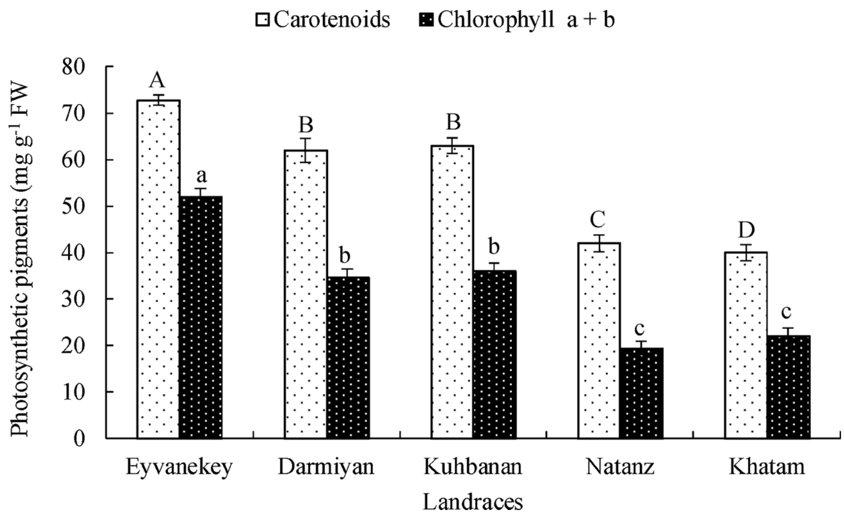

Fig. 8. The contents of photosynthetic pigments in five selected cumin landraces. Means within each character followed by the same letter are not significantly different according to Duncan at 0.05 probability level.

also determined by path analysis. The direct effects of SWM and days to maturity (60% and 35%, respectively, Table 5) on essential oils yield were more than their indirect effects (14% and 23%, respectively, Table 5); hence selection for essential oil yield through these characters would be effective. In another study, the 1000-seed weight had a significant and positive correlation with essential oil yield (Bahraminejad et al., 2011). Direct selection of essential oil yield as a polygenic character is not effective for its improvement, while, it can be improved by selection of other traits. Based on the results of correlation coefficient, stepwise regression, and path coefficient analysis, two characters including SWM and days to maturity were important for improving cumin essential oil yield. This is a good way if the objective is to increase the essential oil yield per hectare for extraction. In other words, a positive selection of SWM and days to maturity are strongly recommended based on the results of regression and path analysis. When there is no environmental stresses, the long duration of maturity was recommended for production and accumulation of secondary metabolites and essential oils (Bahmani et al., 2015). In a study on the path analysis of cumin, the number of seeds per umbrella and number of umbel per plant had the highest direct effect on the seed yield (Bahraminejad et al., 2011).

The morpho-phenological data are good for genotypes clustering because facility of measurement and being an indicator of variations in genome coding regions and environmental effects. Morpho-phenological characters include environmental and genetic effects (Labra et al., 2004; Bahmankar et al., 2016). Four morpho-phenological clusters were obtained by cluster analysis indicating genetic variation among studied landraces (Fig. 4). Landraces belonging to different geographic regions located in the same group for example, Rafsanjan landrace from Kerman province with a semi-arid climate and a mean annual rainfall of 126 ml is located in the same group (second group) with a Jat landrace of Golestan province with a mean rainfall about 315 ml (Fig. 4, Table 1). So, it was concluded that the geographical factor is not the only cause of genetic variation and it is not the reason for genetic distance and closeness of landraces. This is consistent with correlation results so that only altitude possessed a negative correlation with BWM

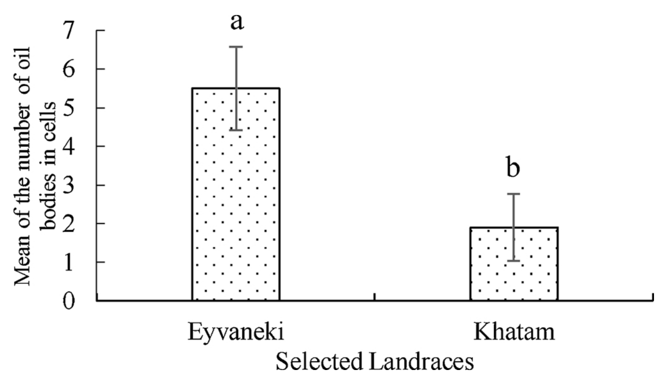

Fig. 10. The number of oil bodies in a cell of the selected cumin landraces. Landraces with one common letter at least are not significantly different at the 5% level.

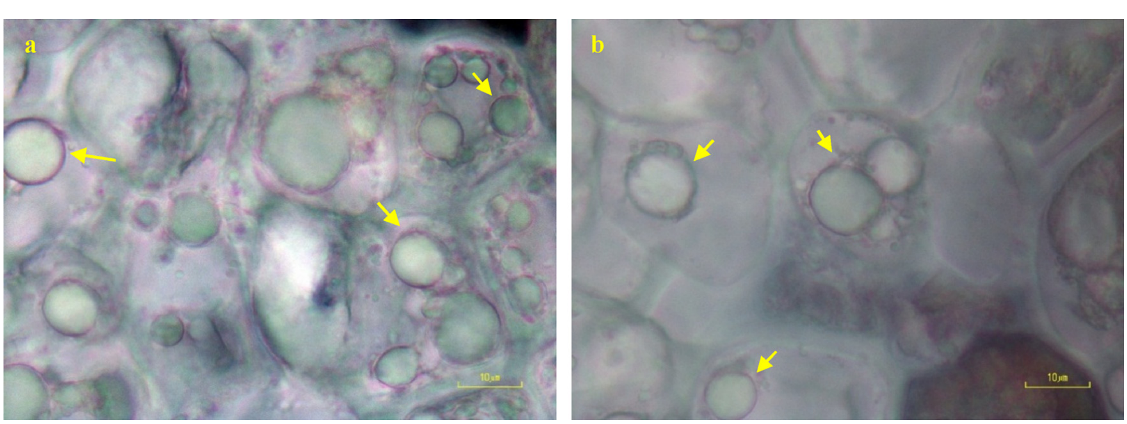

Fig. 9. Oil bodies in the cross-sections cell of the seeds of Eyvanaki landrace (a) and Khatam landrace (b). Arrows indicate oil bodies.

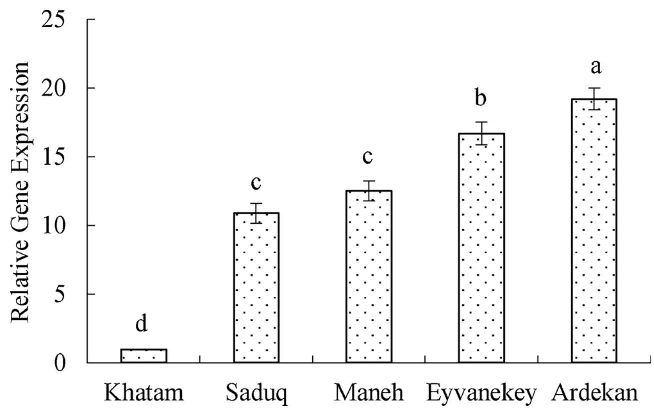

Fig. 11. Relative expression of limonene synthase gene in five selected cumin landraces based on Duncan method. Landraces with one common letter at least are not significantly different at the 5% level.

and 1000-seed weight, while the latitude and longitude had no correlation with morphological characters. The second and third groups included landraces with the highest essential oil yield and days to maturity (Table 6). While landraces of the fourth group had least days to maturity and essential oil yield. So, hybrids obtained from the crosses of landraces located in the fourth group with landraces located in the second or third groups will be superior plants in terms of essential oil yield. Genetic variation plays a main role in plant breeding because hybrids obtained from the lines with more genetic variation show more heterosis compared to convergent races (Bahmankar et al., 2016). Other researchers reported that the cumin landraces originated from various regions were difference in terms of yield and related characters (Bahraminejad et al., 2011; Moghaddam and Pirbalouti, 2017). It is reported that the cumin essential oil content is mainly influenced by genotype (Bettaieb et al., 2011; Rebey et al., 2012; Moghaddam and Pirbalouti, 2017). On the other hand, some researchers reported that the essential oils production is a mechanism for adapting to changing environmental conditions (Sangwan et al., 2001).

4.2. Phytochemical compounds

Monoterpenes and oxygenated monoterpenes were the major compounds in the cumin essential oils. In other studies, oxygenated monoterpenes and monoterpenes were reported as the most important chemical compounds of cumin essential oils (El-Hamidi and Ahmed, 1966; Raghavan, 2006; Dubey et al., 2017; Moghaddam and Pirbalouti, 2017).

The tested landraces varied significantly for essential oil quality. The number of identified essential oil compounds in landraces was ranged from 25 (in the Natanz landrace) to 50 (in the Jat, Saduq, and Sorkheh landraces, data not shown). In the present study, 50 compounds were identified in cumin essential oil, which covers approximately 98% of essential oil content (data not shown). In another study, 19 components in Chinese landraces, 41 components in Egyptian landraces and 29 components in Indian landraces were determined by GC-MS analysis (Dubey et al., 2017). Different results for essential oil compositions may be due to factors such as the sensitivity of system used or method adapted for analysis (Bettaieb et al., 2011; Dubey et al., 2017). Many internal and external factors including genotype, geographical location, climatic conditions, growing season, and the methods used for essential oils extraction affect the chemical compositions of essential oil (Bahmani et al., 2015; Dubey et al., 2017). According to essential oil compositions, the cumin landraces were clustered in two chemotypes including low cuminal / high monoterpenes and high cuminal / low monoterpenes, indicating a significant difference in the essential oil quality. Khansar landrace with the highest cuminal content (42.30%) was located in the second chemotype (Fig. 5, Table 7). On the other hand, Khansar landrace was identified as one of the late maturing landraces (117.67 d, Table 2). Many studies reported a positive and significant relationship between essential oil content and cuminal content in cumin (Dubey et al., 2017; Moghaddam and Pirbalouti, 2017). The high contents of essential oil and cuminal and low content of monoterpenes have been reported in late maturing landraces (Dubey et al., 2017). The phytochemical profiles of the second chemotype showed that the cuminal content (35.21%) in its essential oils was high, while monoterpenes (13.98%) and sesquiterpenes (0.23%) contents were low (Table 7 and Fig. 5). In the current study, cuminal content (27.97%) was higher than monoterpenes content (20.96%). There was a significant and negative correlation (r = -0.53*) between monoterpenes content and cuminal content. A negative relationship between monoterpenes content and the cuminal content was reported in cumin (Moghaddam and Pirbalouti, 2017). The content of cumin aldehyde (cuminal) in Tunisian cumin landraces was higher than monoterpenes content (Hajlaoui et al., 2010). However, other studies reported that monoterpenes content was higher compared to cumin aldehyde in cumin landraces (Gachkar et al., 2007; Mohammadpour et al., 2012). Cuminal possesses a pleasant smell and it contributes to the special aroma of its spice. The cuminal is commercially applied in fragrance, and cosmetics industries (Pandey et al., 2015; Sharma et al., 2016). Cumin seeds with high cuminal content are more desirable compared to seeds with high monoterpenes content in local and international markets (Dubey et al., 2017). Therefore, the landraces located in the second chemotype especially Khansar landrace could be introduced as the best Iranian landrace in terms of essential oil quality for applying in the pharmaceutical industry. Mean of sesquiterpenes content in studied cumin landraces was 0.98% and ranged from 0.11% (Rafsanjan landrace) to 4.08% (Ardekan landrace). In other studies, sesquiterpenes content of essential oil in Iranian cumin was less than 1% (Moghaddam et al., 2015; Moghaddam and Pirbalouti, 2017). The antibacterial properties of cumin are related to cuminal, limonene, γ-Terpinene, and β-Pinene (Iacobellis et al., 2005). These compounds are used to produce antibacterial drugs and overcome the resistance of microbe to traditional antibiotics (Bettaieb et al., 2011). Ardekan had the highest content of γ-Terpinene (17.10%) and β-Pinene (9.95%, Table 7). In other studies, γ-Terpinene, cuminal, and β-Pinene were reported as the major compositions of cumin essential oils (Hajlaoui et al., 2010; Xie et al., 2011; Moghaddam and Pirbalouti, 2017). In another study, 21 compounds were detected in the cumin essential oils and γ-Terpinene (24.30%), cuminaldehyde (cuminal, 21.70%), p-cymene (16.50%), β-Pinene (13.70%), Safranal (12.93%), and cumin alcohol (2.40%) were the major compounds (Moghaddam et al., 2015). γ-terpinene, β-pinene, p-cymene, and cuminaldehyde were reported as the main chemical compositions of Jat (Kazemi et al., 2018) and Rafsanjan (Mortazavian et al., 2018) landraces. The quality of cumin essential oil is influenced by factors such as production region, harvesting season, extraction method, and storage condition (Xie et al., 2011; Moghaddam and Pirbalouti, 2017;). In the current study, Neronine alkaloid with the chemical formula of $C_{18}H_{21}NO_6$ (Fig. 7) and molecular weight of 347 g/mol was observed only in the Jat landrace originated from Golestan province and it was about 2.02% of essential oil (Table 7). Up to now, this alkaloid with anti-cancer, antiviral, and disinfectant properties has not been reported in cumin and has been reported in the *Pancratium maritimum* belong to Amaryllidaceae family (Clark et al., 1975; Bessa et al., 2016). Since the tested landraces were planted under the same climatic situation, all observed differences are related to their genetic structure. The uniformity of environmental conditions helps to recognize the effect of genetic variation on the phytochemical characters (Kruger et al., 2002). Xie et al. (2011) reported that cumin landraces originated from the different regions had different content of main essential oil components. Aldehyde / γ-Terpinene, and γ-Terpinene/ 1-Phenyl-1-Ethanediol chemotypes have been reported in Tunisian and Indian cumin landraces, respectively (Bettaieb et al., 2011). Also, γ-Terpinene (Viuda-Martos et al., 2007) and cuminaldehyde (cuminal, Beis et al., 2000) chemotypes have been reported in cumin landraces.

In the current study, the dendrogram obtained using morpho-phenological characters was different with the dendrogram obtained using phytochemical properties. Khadivi-Khub et al. (2014) observed that the dendrogram obtained using morphological characters was mostly differenced from the one based on phytochemical compositions, in which most samples originated from the same location were clustered in several subgroups by morphological characters and showed high intraregional variation, while they were placed in the same subgroups by phytochemical compositions in most cases. The disagreement between dendrograms from morphological variables and the main phytochemical compositions could be attributed to a number of reasons; one is higher number of morphological traits than phytochemical compositions, which could cause higher variations (Khadivi-Khub et al., 2014).

4.3. Photosynthetic pigments contents

All terpenes are generated from the C5 precursor's isopentenyl pyrophosphate (IPP), and its isomer dimethyl allyl pyrophosphate (DMAPP, Martin et al., 2003). Dudareva et al. (2004) reported that monoterpenes biosynthesis is affected by mitochondrial function, chlorophyll, and photosynthetic efficiency. Carotenoids are accessory light harvesting pigments that protect photosynthetic pigments from photo-damage. Eyvanekey landrace with the highest essential oil yield (14.33 kg ha^{-1}) had the highest contents of carotenoids (72.8 mg g^{-1} FW) and Chl a + b (52 mg g^{-1} FW). Khatam with least essential oil yield (1 kg ha^{-1}) had the least contents of carotenoids (40 mg g^{-1} FW) and Chl a + b (22 mg g^{-1} FW, Fig. 8, Table 2). Individual data of essential oil yield possessed high correlation with individual data of carotenoids content ($r = 0.91^{**}$) and Chl a + b content ($r = 0.95^{**}$). Chlorophylls and carotenoids contents had a positive effect on photosynthetic capacity and more photosynthesis resulted in more biosynthesis of hydrocarbons and protein in the plant (Parida et al., 2002).

4.4. Oil body number in the cell

Oil bodies are lipid (mainly triacylglycerols) storage compartments. Triacylglycerols are the major oils used for energy and metabolic substrates during germination and seedling growth (Tzen and Huang, 1992; Shimada and Hara-Nishimura, 2010; Shimada et al., 2018). Eyvanekey landrace [as late maturity (121 d, Table 2) landrace with high essential oil content and yield (2.14% and 14.33 kg ha^{-1}, respectively, Table 2)] had more number of oil body (5.50 no./cell) compared to Khatam landrace [1.90 no./cell, Fig. 9, Fig. 10, as early maturity landrace (105 d, Table 2) with low essential oil content and yield (0.68%, 1 kg ha^{-1}, respectively, Table 2)]. Therefore, it may be concluded that oil body is one of the effective factors in the essential oil synthesis of cumin landraces and they positively affect the essential oils content. Oil bodies may function to provide bioactive secondary compounds (Shimada et al., 2018). CLO4 (CALEOSIN4, a oil body protein) is involved in abscisic acid (ABA) pathways (Kim et al., 2011) and phytohormones such as ABA may induce the expression levels of transcription factors (TFs); subsequently, these phytohormone-responsive TFs bind the promoters of secondary metabolite pathway genes. Environmental conditions such as dark and extreme temperature (heat and cold) induce oil body formation (Gidda et al., 2016), suggesting that leaf oil bodies function in stress responses. Other researchers have also verified that the late-maturing genotypes of medicinal plants had a high capacity of essential oils synthesis (Bahmani et al., 2015; Moghaddam and Pirbalouti, 2017).

4.5. Limonene synthase gene expression

One important regulatory mechanism of secondary metabolite pathways are transcriptional co-regulation of genes involved in such pathways (Salehi et al., 2018a; Salehi et al., 2018b). The qPCR method was used to understand the relationship of monoterpenes content or cuminal content with the expression pattern of limonene synthase gene. Ardekan landrace with the highest monoterpenes content (34.20%) had the highest expression of limonene synthase gene (19. 20 fold) followed by Eyvanekey landrace (16.7 fold) with the highest monoterpenes content (33.95%, Fig. 11, Table 7). The least expression of limonene synthase gene was found in the Khatam landrace (1 fold) with least monoterpenes content (12.80%, Fig. 11, Table 7). There was a significant and positive correlation ($r = 0.97^{**}$) between monoterpenes content and limonene synthase gene expression. This relationship between limonene synthase gene expression and the monoterpenes content of cumin essential oil has been also reported in other studies (Kjonaas and Croteau, 1983; Munoz-Bertomeu et al., 2008; Zarinkamar et al., 2012; Bahmankar et al., 2018). The antibacterial properties of cumin are related to some of the monoterpenes hydrocarbons such as limonene, γ-Terpinene, and β-Pinene (Iacobellis et al., 2005). These compounds are used to produce antibacterial drugs and overcome the microbe's resistance to traditional antibiotics (Bettaieb et al., 2011). It is possible that monoterpenes production protects plants against pathogens and herbivore-feeding deterrents during growth and development (Ibrahim et al. 2008). Also, Munoz-Bertomeu et al. (2008) showed that the protective properties of the transgenic mint plant with limonene gene increased. Limonene synthase gene expression positively affects the monoterpenes pathways which results in increased resistance of the plant.

A negative relationship between monoterpenes content and cuminal content was reported in cumin (Moghaddam and Pirbalouti, 2017). Cumin seeds with high cuminal content are more desirable compared to seeds with the high monoterpenes content in local and international markets (Dubey et al., 2017). A negative relationship between the cuminal content and limonene synthase gene expression (r = -0.55^{*}) was found. Hence, it was concluded that landraces having low expression of limonene synthase gene are favorable for marketing.

5. Conclusions

The cumin landraces were classified into four morpho-phenological groups indicating genetic variation between them. We also observed two different chemotypes (low cuminal / high monoterpenes and high cuminal / low monoterpenes) in Iranian cumin landraces, which verified the existence of a wide genetic variation among studied cumin landraces. Genetic variation can be used in selection and breeding programs for developing desirable cumin cultivars. Evaluation of morpho-phenological and phytochemical characters of cumin landraces in addition to the application in the breeding programs is commercially important. Results showed that SWM and days to maturity are the good morphological indexes for selection of landraces with high-essential oil yield. Phytochemical analysis of landraces showed that terpenes were the main components of cumin essential oil. Neronine alkaloid was identified for the first time in the current study, and Jat landrace is a novel plant source of Neronine alkaloid. Carotenoids and chlorophylls positively affect the essential oil yield. Late maturity landraces had higher content of essential oil compared to early maturity landrace. This result is consistent with the results of Bardideh et al. (2013) and Moghaddam et al. (2015). On the other hand, the oil body number in the cells of the late maturity landrace was significantly more compared to that in the early maturity landrace. Due to the fact that the maturity period is a genetic character, locus related to the maturity period and oil body number may be linkage with each other or controlled by a pleotropic locus. Hence, it was concluded that the number of oil body in the cells of the late maturity landrace was significantly higher compared to that in the early maturity landrace and it positively affected the essential oils synthesis However, since the oil body number has only been studied in two landraces, this is only an idea for future studies. Limonene synthase gene expression had a positive effect on the monoterpenes biosynthesis pathway in cumin.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

Authors gratefully acknowledge the support provided for this survey by grant No: 950503 of the Biotechnology Development Council of the Islamic Republic of Iran. The authors also would like to thank Dr. Maryam Salehi, for her helpful assistance and comments during the experiment.

References

Ali, N., Javidfar, F., Jafari Yazdi, E., Mirza, M.Y., 2003. Relationship among yield components and selection criteria for yield improvement in winter rapeseed. Pak. J. Bot. 35, 167–174.

Bahmani, K., Izadi Darbandi, A., Ramshini, H.A., Moradi, N., Akbari, A., 2015. Agro-morphological and phytochemical diversity of various Iranian fennel landraces. Ind. Crops Prod. 77, 282–294.

Bahmankar, M., Mortazavian, S.M.M., Tohidfar, M., Sadat Noori, S.A., Izadi Darbandi, A., Corrado, G., Rao, R., 2017. Chemical compositions, somatic embryogenesis, and somaclonal variation in cumin. Biomed Res. Int. 2017, 15.

Bahmankar, M., Mortazavian, S.M.M., Tohidfar, M., Sadat Noori, S.A., Izadi Darbandi, A., Salehi, M., Rao, R., 2018. Physio-biochemical characters, embryo regeneration and limonene synthase gene expression in cumin. Ind. Crops Prod. 121, 195–205.

Bahmankar, M., Nabati, D.A., Dehdari, M., 2014. Correlation, multiple regression and path analysis for some yield-related traits in safflower. J. Biodivers. Environ. Sci. 4, 111–118.

Bahmankar, M., Nabati, D.A., Dehdari, M., Ramazani, S.H.R., 2016. Genetic Variation and Phylogenetic Relationships of Safflower using Morpho-Phenological Markers. Electronic. J. Biol. 1, 7–12.

Bahraminejad, A., Mohammadinejad, G., 2013. Use of microsatellite markers for molecular characterization of cumin (*Cuminum cyminum* L.) ecotypes. Iran. J. Genet. Plant Breed 2, 35–41.

Bahraminejad, A., Mohammadi-Nejad, G., Khadir, M.A., 2011. Genetic diversity evaluation of cumin (*Cuminum cyminum* L.) based on phenotypic characteristics. Aust. J. Crop Sci. 5, 304–310.

Bardideh, K., Kahrizi, D., Ghobadi, M.E., 2013. Character association and path analysis of black cumin (*Nigella sativa* L.) genotypes under different irrigation regimes. Not. Sci. Biol. 5, 104–108.

Barra, A., 2009. Factors affecting chemical variability of essential oils: a review of recent developments. Nat. Prod. Commun. 4, 1147–1154.

Beis, S.H., Azcan, N., Ozek, T., Kara, M., Baser, K.H.C., 2000. Production of essential oil from cumin seeds. Chem. Nat. Compd. 36, 265–268.

Bessa, C., Andrade, J.P., Oliveira, R.S., Domingos, E., Santos, S., Romao, W., Bastida, J., Borges, W.S., 2016. Identification of alkaloids from *Hippeastrum aulicum* (Ker Gawl.) Herb. (Amaryllidaceae) using GC-MS and ambient ionization mass spectrometry (PS-MS and LS-MS). J. Braz. Chem. Soc. 28, 819–830.

Bettaieb, I., Bourgou, S., Sriti, J., Msaada, K., Limam, F., Marzouk, B., 2011. Essential oils and fatty acids composition of Tunisian and Indian cumin (*Cuminum cyminum* L.) seeds: A comparative study. J. Sci. Food Agric. 91, 2100–2107.

Clark, R.C., Warren, F.L., Pachler, K.G.R., 1975. Alkaloids of the amaryllidaceae: Brunsvigine: NMR, ORD/CD and mass spectrometry, degradation and interconversion studies. Tetrahedron 31, 1855–1859.

Derakhshan, S., Sattari, M., Bigdeli, M., 2010. Effect of cumin (*Cuminum cyminum*) seed essential oil on biofilm formation and plasmid integrity of *Klebsiella pneumoniae*. Pharmacogn Mag 6, 57–61.

Dubey, P.N., Saxena, S.N., Mishra, B.K., Solanki, R.K., Vishal, M.K., Singh, B., Sharma, L.K., John, S., Garwal, D.A., Yogi, A., 2017. Preponderance of cumin (*Cuminum cyminum* L.) essential oil constituents across cumin growing Agro-Ecological Sub Regions. India. Ind. Crops Prod. 95, 50–59.

Dudareva, N., Picheresky, E., Gershenzon, J., 2004. Biochemistry of plant volatiles. Plant Physiol. 134, 1893–1902.

El-Hamidi, A., Ahmed, S.S., 1966. The effect of plant age on content and composition of dill essential oil *Anethum graveolens* L. Pharamazie 21, 438–439.

Gachkar, L., Yadegari, D., Rezaei, M.B., Taghizadeh, M., Alipoor Astaneh, S., Rasooli, I., 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem. 102, 898–904.

Gidda, S.K., Park, S., Pyc, M., Yurchenko, O., Cai, Y., Wu, P., Andrews, D.W., Chapman, K.D., Dyer, J.M., Mullen, R.T., 2016. Lipid Droplet-Associated Proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells. Plant Physiol. 170, 2052–2071.

Hajlaoui, H., Mighri, H., Noumi, E., Snoussi, M., Trabelsi, N., Ksouri, R., Bakhrouf, A., 2010. Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: A high effectiveness against Vibrio spp. strains. Food Chem. Toxicol. 48, 2186–2192.

Hashemian, N., Ghasemi Pirbalouti, A., Hashemi, M., Golparvar, A., Hamedi, H., 2013. Diversity in chemical composition and antibacterial activity of essential oils of cumin (*Cuminum cyminum* L.) diverse from northeast of Iran. Aust. J. Crop Sci. 7, 1752–1760.

Hemeda, H.M., Klein, B.P., 2006. Effects of Naturally Occurring Antioxidants on Peroxidase Activity of Vegetable Extracts-Hemeda. J. Food Sci. 55, 184–186.

Iacobellis, N.S., Pietro, L.C., Capasso, F., Senatore, F., 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J. Agric. Food Chem. 53, 57–61.

Ibrahim, M., Kainulainen, P., Aflatuni, A., 2008. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. Agric. Food Sci. 10, 243–259.

Jalali-Heravi, M., Zekavat, B., Sereshti, H., 2007. Use of gas chromatography–mass spectrometry combined with resolution methods to characterize the essential oil components of Iranian cumin and caraway. J. Chromatogr. A 1143, 215–226.

Jirovetz, L., Buchbauer, G., Stoyanova, A.S., Georgiev, E.V., Damianova, S.T., 2005. Composition, quality control and antimicrobial activity of the essential oil of cumin (*Cuminum cyminum* L.) seeds from Bulgaria that had been stored for up to 36 years. Food Sci. Technol. Int. 40, 305–310.

Karimzadeh-Asl, K., Sefidkon, F., Ebrahimi, M., Farajpour, M., Marefatzadeh, M., 2015. Sequential path analysis of effective characters on the essential oil yield of *Dracocephalum moldavica* L. J. Essent. Oil Bear. PL. 18, 702–712.

Kazemi, H., Mortazavian, S.M.M., Gorbani-Javid, M., 2018. Breeding cumin landraces (*Cuminum cyminum* L.) for drought tolerance based on physiological and genetical traits. J. Appl. Res. Med. Aromat. Plants 9, 78–90.

Khadivi-Khub, A., Salehi-Arjmand, H., Hadian, J., 2014. Morphological and phytochemical variation of *Satureja bachtiarica* populations from Iran. Ind. Crops Prod. 54, 257–265.

Kim, Y.Y., Jung, K.W., Yoo, K.S., Jeung, J.U., Shin, J.S., 2011. A stress-responsive caleosin-like protein, AtCLO4, acts as a negative regulator of ABA responses in Arabidopsis. Plant Cell Physiol. 52, 874–884.

Kjonaas, R., Croteau, R., 1983. Demonstration that limonene is the first cyclic intermediate in the biosynthesis of oxygenated p-menthane monoterpenes in *Mentha piperita* and other *Mentha* species. Arch. Biochem. Biophys. 220, 79–89.

Kruger, H., Sabine, B., Wetzel Zeiger, B., 2002. The chemical variability of *Ocimum* species. J. Herbs Spices Med. Plants 9, 335–344.

Labra, M., Miele, M., Ledda, B., Grassi, F., Mazzei, M., Sala, F., 2004. Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. Cultivars. Plant Sci. 167, 725–733.

Lal, R.K., 2007. Associations among agronomic traits and path analysis in fennel (*Foeniculum vulgare* Miller). J. Sustain. Agr. 30, 21–29.

Li, P., Wang, Y., Chen, Y., Zhang, S., 2009. Genetic diversity and association of ISSR markers with the eleostearic content in tung tree (*Vernicia Fordii*). Afr. J. Biotechnol. 8, 4782–4788.

Li, R., Jiang, Z.T., 2004. Chemical composition of the essential oil of *Cuminum cyminum* L. from China. Flavour Frag. J. 19, 311–313.

Lichtenthaler, H.K., Buschman, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV–vis spectroscopy. Curr. Protoc. Food Anal. Chem. F4.3.1–F4.3.8.

Lucker, J., Schwab, W., van Hautum, B., Blaas, J., van der Pals, L.H.W., Bouwmeester, H.J., Verhoeven, H.A., 2004. Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. Plant Physiol. 134, 510–519.

Martin, V.J.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D., 2003. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat. Biotechnol. 21, 96–802.

Moghaddam, M., Khaleghi Miran, S.N., Ghasemi Pirbalouti, A., Mehdizadeh, L., Ghaderi, Y., 2015. Variation in essential oil composition and antioxidant activity of cumin (*Cuminum cyminum* L.) fruits during stages of maturity. Ind. Crops Prod. 70, 163–169.

Moghaddam, M., Pirbalouti, A.G., 2017. Agro-morphological and phytochemical diversity of Iranian *Cuminum cyminum* accessions. Ind. Crops Prod. 99, 205–213.

Mohammadpour, H., Moghimipour, E., Rasooli, I., Fakoor, M.H., Alipoor Astaneh, S., Shehni Moosaie, S., Jalili, Z., 2012. Chemical composition and antifungal activity of *Cuminum cyminum* L. essential oil from alborz mountain against *Aspergillus* species. Jundishapur J. Nat. Pharm. Prod. 7, 50–55.

Mortazavian, S.M.M., Safari, B., Sadat Noori, S.A., Foghi, B., 2018. Evaluation of diverse cumin (*Cuminum cyminum* L.) ecotypes for seed yield under normal and water stress condition. J. Agr. Sci. Tech. 20, 359–372.

Munoz-Bertomeu, J., Ros, R., Arrillaga, I., Segura, J., 2008. Expression of spearmint limonene synthase in transgenic spike *lavender* results in an altered monoterpene composition in developing leaves. Metabm Eng. 10, 166–177.

Pandey, S., Patel, M.K., Mishra, A., Jha, B., 2015. Physio-biochemical composition and untargeted metabolomics of cumin (*Cuminum cyminum* L.) make it promising functional food and help in mitigating salinity stress. PLoS One 10, 1–25.

Panico, A.M., Cardile, V., Garufi, F., Puglia, C., Bonina, F., Ronsisvalle, G., 2005. Protective effect of Capparis spinosa on chondrocytes. Life Sci. 77, 2479–2488.

Parida, A., Das, A.B., Das, P., 2002. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. Plant Biol. 45, 28–36.

Pfaffi, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Raghavan, S., 2006. Handbook of spices, seasonings, and flavourings. CRC Press, Boca Raton, pp. 330 Taylor and Francis Group.

Rebey, I.B., Jabri-Karoui, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., Marzouk, B., 2012. Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Ind. Crops Prod. 36, 238–245.

Safari, B., Mortazavian, S.M.M., Sadat-Noori, S.A., Foghi, B., 2015. Effect of water stress on yield and yield components of cumin (*Cuminum cyminum* L.) ecotypes. J. Plant

Physiol. Breed. 5, 51–61.
Salehi, M., Karimzadeh, G., Naghavi, M.R., Naghdi Badi, H., Rashidi Monfared, S., 2018a. Expression of artemisinin biosynthesis and trichome formation genes in five *Artemisia* species. Ind. Crops Prod. 112, 130–140.
Salehi, M., Karimzadeh, G., Naghavi, M.R., Naghdi Badi, H., Rashidi Monfared, S., 2018b. Expression of key genes affecting artemisinin content in five *Artemisia* species. Sci. rep. 8, 12659.
Sangwan, N., Yadav, U., Sangwan, R., 2001. Molecular analysis of genetic diversity in elite India cultivars of essential oil trade types of aromatic grasses (*Cymbopogon* species). Plant Cell Rep. 20, 437–444.
Sharma, L.K., Agarwal, D., Rathore, S.S., Malhotra, S.K., Saxena, S.N., 2016. Effect of cryogenic grinding on volatile and fatty oil constituents of cumin (*Cuminum cyminum* L.) genotypes. J. Food Sci. Tech. 53, 2827–2834.
Shimada, T.L., Hara-Nishimura, I., 2010. Oil-body-membrane proteins and their physiological functions in plants. Biol. Pharm. Bull. 33, 360–363.
Shimada, T.L., Hayashi, M., Hara-Nishimura, I., 2018. Membrane dynamics and multiple functions of oil bodies in seeds and leaves. Plant physiol. 176, 199–207.
Siloto, R.M.P., Findlay, K., Lopez-Villalobos, A., Yeung, E.C., Nykiforuk, C.L., Moloney, M.M., 2006. The accumulation of oleosins determines the size of seed oilbodies in Arabidopsis. Plant Cell. 18, 1961–1974.
Sowbhagya, H.B., Sathyendra Rao, B.V., Krishnamurthy, N., 2008. Evaluation of size reduction and expansion on yield and quality of cumin (*Cuminum cyminum*) seed oil. J. Food Eng. 84, 595–600.
Tzen, J.T.C., Huang, A.H.C., 1992. Surface Structure and Properties of Plant Seed Oil Bodies. J. Cell Biol. 117, 327–335.
Viuda-Martos, M., Ruiz Navajas, Y., Fernandez-Lopez, J., Perez-Alvarez, J.A., 2007. Chemical composition of the essential oils obtained from some spices widely used in mediterranean region. Acta Chim. Slov. 54, 921–926.
Xie, X.G., Abulimiti, Y., Ma, Q.L., Hajiakber, A., 2011. Study on chemical compositions and biological characteristics of *Cuminum cyminum* L. produced in Hotan of Xinjiang prvince. Med. Plant 2, 62–64.
Zarinkamar, F., Ghannadnia, M., Haddad, R., 2012. Limonene synthase gene expression under different concentrations of manganese in *Cuminum cyminum* L. Afr. J. Plant Sci. 6, 203–212.