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Evaluation of Yield and Phytochemical Content of Different Iranian Garlic (*Allium sativum* L.) Ecotypes

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ABSTRACT

Due to the development of garlic cultivation, quantitative and qualitative evaluations of garlic ecotypes in different regions are important for breeding purposes. In this study, some vegetative and phytochemical traits of eight Iranian garlic ecotypes were assayed in a RCBD with three replications at Darab Agricultural Research Station, Iran during 2017-2018 growing season. The results showed that there is a significant difference among garlic ecotypes in terms of studied traits ($P < 0.01$). In general, Tarom and Tafresh ecotypes showed the highest plant height, number of leaves, length of garlic leaves, leaf width, fresh weight, dry weight and the highest bulb diameter. Highest dry weight of garlic bulb (g) per plant was obtained in Darab (79.0 g), Tarom (75.5 g), and Talesh (75.0 g) ecotypes, with no significant difference among them ($P \geq 0.01$). Hamedan and Kerman ecotypes contained higher allicin content, alliin content, TPC, TFC, and TAC than the other ecotypes. Cluster analysis divided ecotypes into three distinct groups. Talesh and Hamedan ecotypes had the lowest similarity (0.34) and Darab and Tafresh ecotypes had the highest similarity (0.97). It can be concluded that Tarom, Tafresh, Hamedan, and Kerman can be recommended for cultivation depending on the quantitative (Tarom and Tafresh) and qualitative (Hamedan and Kerman) goals of cultivation.

Abbreviations

ANOVA: Analysis of variance, CRBD: Complete randomized block design, FAO: Food and agriculture organization, FCR: Folin-Ciocalteu reagent, GAE: Gallic acid equivalent, HPLC: high-performance liquid chromatography, SAS: Statistical analysis system, TAC: Total antioxidant capacity, TPC: Total phenolic content, TFC: Total flavonoid content, QE: quercetin equivalents, FRAP: Ferric reducing the ability of plasma.

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Introduction

Garlic (*Allium sativum* L.) is one of the first domesticated plants. Evidence from garlic cultivation is found in Egypt in 3200 years B.C (Ammarellou, 2017). The global production of garlic is about 28.5 million tons from a cultivated area of 1,546,741 ha. China, India, Bangladesh, South Korea, Egypt, and Spain are the biggest garlic producer in the world and Iran with 4734 ha cultivation area and 58278 tons of production has the 27th rank in the world (FAO, 2018).

Garlic has been known as a healthy herb for centuries and is known as food, spice and medicine. This vegetable plays an important role in diet and medicine around the world (Füstös and Kovács, 2014). Garlic is an important part of the Mediterranean, European and Asian diets as a source of food, as well as a vegetable medicine used to treat various types of diseases. Several anti-fungal, antibacterial, antiviral, antithrombotic, antitumor and hypotensive, hypoglycemic and hypolipidemic properties have been reported for garlic (Ammarellou, 2017). It contains sulfur compounds such as allicin with very beneficial effects on the circulatory and cardiac system (Bahador et al., 2014). Garlic and its products are known as agents for the prevention and treatment of cardiovascular disease, cholesterol metabolism and atherosclerosis as well as cancer (Le Bon and Siess, 2000; Varshney and Budoff, 2016). Many studies confirmed this hypothesis that garlic consumption has a significant effect on reducing serum cholesterol and triglyceride, lowering blood pressure and preventing atherosclerosis, inhibiting platelet aggregation and increasing fibrinolytic activity (Chen et al., 2013).

Garlic propagation is asexual, but it has a wide variety in morpho-physiological and phytochemical characteristics such as bulb color and size, plant height, flowering, flower color, number and size of cloves/bulb, fresh and dry cloves weight, fertility and development of air cloves in inflorescence, length of the growth period, storage property, adaptability to agro-clay conditions and the amount of phytochemical compounds (Abasifar and Dashti, 2015). The results of a study with 38 Iranian garlic genotypes in greenhouse conditions showed that 12 genotypes could be introduced commercially in greenhouses due to the production of several cloves. Also, 26 other genotypes that produce single-cloves can be used for cooking thanks to the easy removal of their skin (Ammarellou, 2017). In another study, 22 genotypes of local garlic were evaluated in the Burgas region of Bangladesh. The results showed that the studied germplasm showed a significant difference in morphological traits such as yield, plant height, number of garlic, garlic, and length and width of garlic cloves (Islam et al., 2004). In another study, 11 genotypes of garlic collected from different regions of Egypt. The results showed that El-Minia, El-Gharbia and El-Behera genotypes had promising results for direct culture (Ahmed et al., 2010). Research results in Allah Abad climate of India to evaluate the seven cultivars of garlic showed that KS-2 cultivar was better in terms of growth, quality and economic performance compared to other varieties (Verma and Thakre, 2018).

Many garlic ecotypes are cultivated in Iran. Due to the high importance of garlic in improving health, special attention has been paid to the cultivation of this product in the country. Therefore, the selection of compatible ecotypes for each region is necessary to obtain high levels of bioactive compounds. The objective of the present study was to investigate the quantitative and qualitative traits of eight garlic ecotypes for (i) evaluation vegetative growth characteristics, yield and yield component (ii) selection the best ecotype in terms of alliin and allicin contents (iii) assessment of total phenolic and flavonoids contents and antioxidant activity of garlic compounds (iv) and selection of the best-adapted garlic ecotype for Darab region.

Materials and Methods

Plant materials and field trials

Eight garlic ecotypes collected from different regions of Iran were used in this study (Fig. 1). The details of these regions are given in Table 1.

The experiment was performed at Bakhtagerd farm (28°7´ N, 49°36´E, 1150 m altitude) of Darab Agricultural Research Station during the 2017-2018 cropping season. The average annual temperature of Darab is 23 °C with minimum and maximum temperatures of -3 and 48 °C, respectively. The long-term average (30 years) of rainfall for this area is 270 mm with a minimum of 80 mm and a maximum of 590 mm. In terms of climatic conditions, Darab climate is considered as a semi-warm and semi-arid. The soil physicochemical properties of the Bakhtagard farm are shown in Table 2.

Fig. 1. Geographic distribution map of studied garlic ecotypes collected from different regions of Iran

Table 1. Names, origin and geographical characteristics of the collection sites of eight studied garlic ecotypes

	Ecotype name	Region originated (City/Province)	Longitude	Latitude	Altitude (m)
1	Bashagard	Bashagard/ Hormozhan	57° 54' 7.856"	26° 27' 18.078"	725
2	Darab	Darab/ Fars	54° 33' 10.478"	28° 45' 19.206"	1159
3	Hamadan	Hamadan/Hamedan	48° 30' 53.593"	34° 48' 15.510"	1818
4	Ramhormoz	Ramhormoz/Khuzestan	49° 36' 15.673"	31° 16' 30.674"	166
5	Kerman	Kerman/Kerman	57° 4' 21.405"	30° 17' 4.243"	1764
6	Tafresh	Tafresh/ Markazi	50° 0' 55.300"	34° 41' 37.867"	1909
7	Talesh	Talesh/ Gilan	48° 54' 14.292"	37° 47' 51.047"	59
8	Tarom	Tarom/ Zanjan	48° 57' 12.683"	36° 55' 34.294"	612

Table 2. Soil physicochemical properties of Bakhtagard Station

Cu	Mn	Zn	Fe	K	P	Sand (%)	Clay (%)	Silt (%)	O.C (%)	EC (dS/m)	pH
(mg/kg)											
1.7	13.7	0.66	5.5	205	10.0	15.2	28	55.8	0.81	0.89	8.2

Garlic ecotypes were cultivated in a farm in complete randomized block design (CRBD) with three replications. Each experimental plot was four beds with a length of 3 m, row spacing 30 cm and 7 cm spacing between plants on beds (45 plant/m^2 density). Field management including irrigation, fertilization and control of diseases, pests and weeds were implemented in all stages of crop growth. At the time of sowing (5 November 2017), Nitrogen (80 kg ha^{-1} from ammonium nitrate), phosphorus (100 kg ha^{-1} from ammonium phosphate) and potassium (150 kg ha^{-1} from potassium sulfate) was applied according to the soil test results (Table 2). All plots were harvested at physiological maturity (75% of the top of plants down) on June 18, 2018, and transferred to the laboratory for quantitative and qualitative measurements.

Vegetative and reproductive characteristics

Vegetative traits such as plant height, length and width of stem and leaves, leaves number per plant, the diameter of stem and reproductive traits such as average bulb weight, yield and yield components, fresh and dry weight of the bulbs and aerial parts and percentage of dry matter were measured according to the method of Abasifar and Dashti, (2015).

Phytochemical characteristics

Allicin content

The allicin content was determined according to Baghalian et al. (2005) with some modification. At first, cloves were dried at 55±5 °C in a vacuum oven (Memmert, Germany) and were powdered in a grinder. The powder was used to prepare the test solution. After the first centrifugation (30 min, 6000 rpm), the supernatant was suspended in 25 mL volume using solution A (a volumetric mixture of formic acid and methanol (40:60)) and centrifuged again then filtered (the stock solution). The stock solution was used to prepare the test solution. Finally, 20 µL of test solution was used for injection into HPLC (Agilent Infinity 1260, USA), equipped with a Zorbax Eclipse Plus C18 column (150 mm × 4.6 mm; particle size 3 µm) and a DAD UV–Visible detector. The mobile phase was methanol–water (50:50) at a flow rate of 0.7 mL/min. Peaks were detected at 254 nm and checked for peak purity using the Chemstation software and DAD detector). The percentage of allicin in the test solution was calculated from the following formula (Baghalian et al., 2005):

$$Allicine = \frac{s_1 m_2 * 22.75}{s_2 m_1}$$

m_1 = the amount of garlic powder used

m_2 = the amount of butylated para-hydrogen beta-benzoate

s_1 = allicin curve surface

s_2 = surface area under the curve for butyl parahydroxybenzoate

Alliin content

The method of Iberl et al. (1990) with some modification was used to measure the content of alliin. Garlic powder (800 mg) rinsed in l0 mL MeOH + 20 mL 0.1 % formic acid in H_2O, then it was shake vigorously or homogenization with a blender; 5 mm at room temperature. The mixture centrifuged for 20 min, in 15000 rpm, at 4°C. Supernatant used for derivatization procedure. Supernatant (200 µL) mixed with 400 µL l-o-phthaldialdehyde in MeOH + 100 µL of tert.-butylthiol in MeOH + 100 µL borate buffer, pH 9.5. Following shaking for 1 min at room temperature, the filtered extract was used for any HPLC determination of alliin. Solutions used for derivatization were 0.14 M o-phthaldialdehyde in methanol (freshly prepared), 1.11 M tert. -butylthiol in methanol, and 0.33 M boric acid, adjusted to pH 9.5 by 4 M sodium hydroxide.

Quantitative determination of alliin by HPLC

For analysis, we used Unicam-Crysat-200, England HPLC system. The analytical column which was a Spherisorb ODS-2 (3 pm) column, 4.0 mm id, with a length of 125 mm. For quantitative determinations a 1 mM solution of L- (+)-alliin was used as the external standard applying the sample preparation and derivatization procedure. Detection wavelength was 337 nm. Eluent was 45 mM phosphate buffer (pH 7.15): 1,4-dioxane : acetonitrile : tetrahydrofuran = 70 : 22.5 : 6 : 1.5. Flow rate was 0.7 mL/mm. The associated peak of alliin shows retention time of 13.6 min.

Total phenolic content (TPC)

The method of Fukumoto and Mazza (2000) with some modifications, based on calorimetrically with the Folin–Ciocalteu (FC) reagent, was used for the determination of TPC content. The reaction mixture contained 500 μL of extract with a dilution of 0.1%, 2.5 mL of freshly prepared 0.2 M FC reagent and 2 mL of sodium carbonate solution. To complete the reaction, the mixture was kept in the dark under ambient conditions for 30 min. The absorbance of the resulting solution was measured at 760 nm in a UV–Vis spectrophotometer (model 8453 Hewlett Packard, Agilent Technologies, USA).

Total flavonoid content (TFC)

TFC in the investigated extracts was determined spectrophotometrically, based on the formation of complex flavonoid-aluminum with the absorptivity maximum at 430 nm (Bozin *et al.*, 2008). One mL of diluted samples were separately mixed with 1 mL of 2% $AlCl_3$ - $6H_2O$. After incubation at room temperature for 15 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoids content was expressed as mg of quercetin equivalents (QE) per g of dried extract, by using a standard curve.

Total antioxidant capacity (TAC)

Preparation of aqueous garlic extract

Garlic extract, was prepared according to the method described by Johnson et al. (2016), as follows; 100 g of garlic was taken after removal of their outer skin and cut into small pieces by the sterile scalpel. The small pieces were blended with 200 mL sterile distilled water using sterile blender for 5 min at medium speed. The macerates were filtered using sterile funnel and Whatman filter paper. The filtered extract was used for later analyses.

Preparation of methanolic garlic extract

Two grams of chopped fresh garlic was extracted with 15 mL of 70% methanol under magnetic stirring for 2 h at room temperature (ca. 24 °C). The extract was centrifuged at 4000 rpm for 20 min, and the supernatant was filtered through polycarbonate 0.4 μm pore size membrane (GE Water & Process Technologies, Trevose, PA). All extractions were performed in 24-27 °C. Finally, the extractions were stored in the darkness at 4 °C within 1 h (Lu et al., 2011).

Ferric reducing antioxidant power (FRAP)

FRAP is mainly based on the absorbance (593 nm) value, which increases due to the formation of tripyridyl-S-triazine complexes with Fe2þ [TPTZ-Fe (II)] in the presence of a reducing agent. For the determination of the antioxidant activity, the FRAP reagent (1.5 mL) was mixed with 100 mL of deionized water and 100 mL of the garlic extracts, Trolox standard, or control [70:30 methanol: water (v/v)]. The reaction mixture was kept for 4 min at room temperature followed by absorbance at 593 nm (Rasul Suleria et al., 2012).

Data analysis

Data analysis was performed using SAS software (Ver 9.4). LSD test was used to determine the comparison of the means ($p < 0.01$). To grouping of ecotypes, according to the characteristics, the similarity matrix of the measured data was evaluated using the Ward method (Ward, 1963) in SAS software (Ver. 9.4), and finally, the dendrogram image was displayed.

Results

Vegetative traits

Means comparison of vegetative characteristics of studied garlic ecotypes are shown in Table 3. There was a significant difference in plant height. The Tarom and Tafresh ecotypes showed the highest plant height of 86.5 and 84.23 cm in comparison to other ecotypes. The lowest plant height was observed in Kerman, Darab and Ramhormoz ecotypes (69.23, 67.66 and 66.66 cm), respectively. The data are shown in Table 3 demonstrated the difference found in the number of leaves among different ecotypes. The highest number of leaves per plant (14.43) was observed in Tarom ecotype and the lowest number was obtained in Bashagard, Kerman and Ramhormoz ecotypes (7.65, 7.93 and 8.33), respectively. The highest length of garlic leaves was observed in Tafresh, Tarom and Hamedan ecotypes with 55.66, 55.33 and 54.66 cm, respectively. The lowest leaf length (35.66 cm) was observed in Ramhormoz ecotype. The results showed that the Tafresh, Darab and Tarom ecotypes had the highest leaf width (3.61, 3.13 and 3.15 cm, respectively), and the minimum leaf width was detected in Ramhormoz ecotype (1.65 cm) (Table 3). The highest and the lowest stem diameter were observed in Darab and Ramhormoz ecotypes with 11.3 and 1.04 mm, respectively (Table 3). Tafresh, Talesh, Darab and Tarom ecotypes showed the highest bulb diameter with 75, 74.8, 72.33 and 71 mm, respectively (Table 3). The Darab and Ramhormoz showed the most potential for the production of cloves per plant (19.66 and 18.33), respectively (Fig 2. a and h), compared to the other ecotypes (Table 3).

Table 3. Means comparison of vegetative characteristics of eight Iranian garlic ecotypes in Darab region

Ecotypes	Plant height (cm)	Number of leaves per plant	Leaf width (cm)	Leaf length (cm)	Number of garlic (m2)	Number of cloves/ plant	Bulb diameter (mm)	Stem diameter (mm)
Darab	67.66d	12.33bc	3.52ab	53.43bc	14.67ab	19.67a	72.33a	3.11a
Tarom	86.5a	14.43a	3.51ab	55.33a	15.33a	12.33b	71.00a	2.43b
Talesh	82.5b	13.00b	3.36c	52.20c	15.00a	14.00b	74.80a	2.58b
Tafresh	84.23ab	11.66c	3.61a	55.66a	12.00b	12.33b	75.00a	2.45b
Hamadan	75.00d	11.26c	3.11c	54.06ab	14.66ab	11.66b	65.33b	2.51b
Ramhormoz	66.66d	8.33d	1.65e	35.66e	14.33ab	18.33a	53.33c	1.04d
Kerman	69.33d	7.93d	2.00d	40.00d	8.33c	12.00b	45.66d	1.54c
Bashagard	72.33c	7.66d	1.91d	40.60d	7.66c	3.33c	40.90d	1.61c
LSD 5%	2.976	1.073	0.181	1.673	2.737	2.499	5.036	0.357

The means which have at least one common letter do not have significant difference based on LSD test at 5% level.

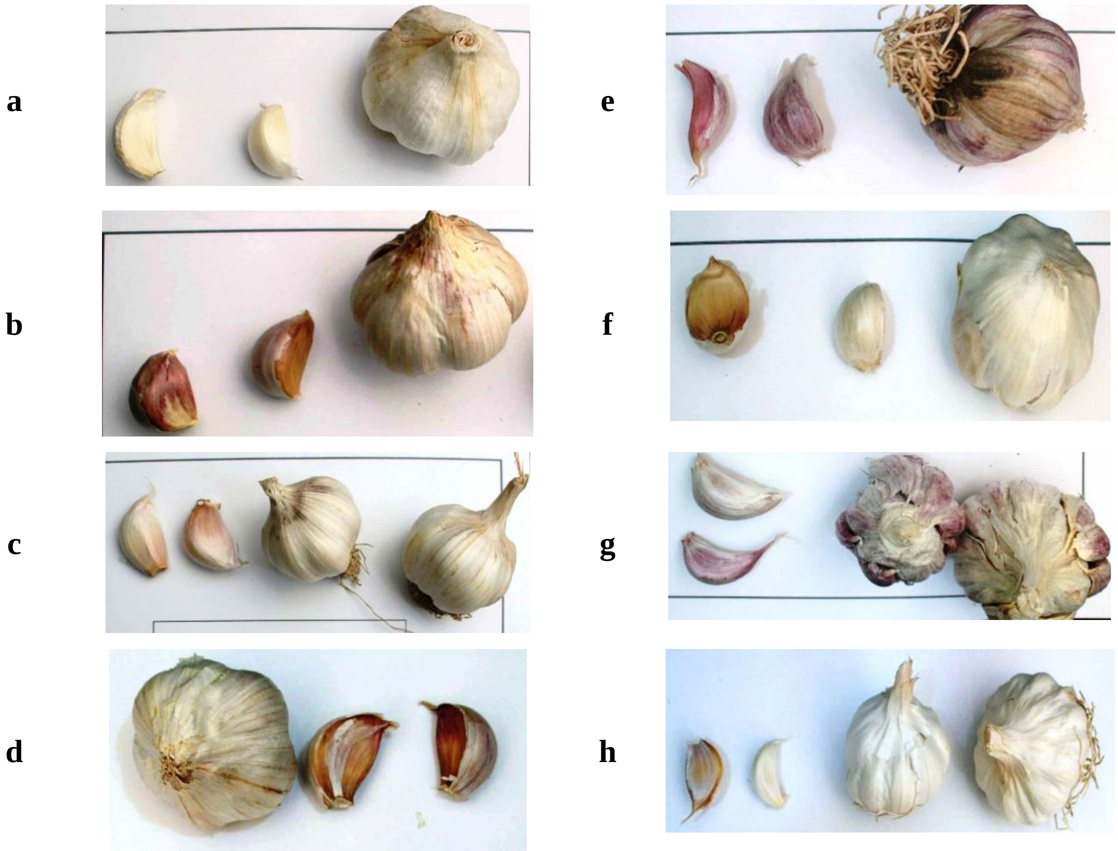

Fig. 2. Pictures of garlic samples collected from different regions of Iran. a = Darab, b = Tafresh, c = Talesh, d = Tarom, e = Kerman, f = Hamedan, g = Bashagard, h = Ramhormoz

Yield and yield components

The difference among garlic ecotypes was significant for yield and yield components (Table 4). As shown in Table 5, the highest cloves fresh weight was obtained in Tarom, Darab, Tafresh and Talesh ecotypes with 1612, 1372, 1319 and 1215 g/m^2, respectively. While the Bashagard, Kerman and Ramhormoz ecotypes showed the lowest cloves fresh weight with 530, 532 and 620 g/m^2, respectively. The highest dry weight was obtained in Darab, Tarom and Tafresh ecotypes with 1131.67, 1128.67, 1002.33 g/m^2, respectively. In contrast, the Bashagard, Kerman and Ramhormoz ecotypes showed the lowest dry weight with 282.33, 338 and 406 g/m^2, respectively (Table 5). Among the studied ecotypes, Darab and Bashagard ecotypes showed the highest (79 g) and lowest (33 g) dry weight of single clove, respectively. Also, the highest (10.33 g) and lowest (1.80 g) dry weight of cloves were obtained in Bashagard and Ramhormoz ecotypes, respectively (Table 5).

Content of allicin and alliin

Studied Iranian garlic ecotype was significantly different ($p < 0.05$) in terms of allicin and alliin content (Table 6). The allicin content ranged between 3.69 and 7.12 mg/g dry matter and alliin were ranged between 2.5 and 5.38 mg/g dry matter. The highest allicin content was obtained in Bashagard (7.12 mg/g dry matter) and Hamedan (6.48 mg/g dry matter) ecotypes and the lowest was observed in Talesh ecotype (3.69 mg/g dry matter) (Table 6). The Hamedan ecotype showed the highest alliin content (5.38 mg/g dry matter) as compared to other ecotypes. The lowest alliin content was obtained in Tafresh and Talesh ecotypes (2.5 and 2.54 mg/g/ dry matter), respectively (Fig. 3, Table 6).

Table 4. Results of ANOVA vegetative, reproductive and phytochemical compounds of different garlic ecotypes

Variables	Effects	*df*	*Mean square*	*F*- value
Plant height	Ecotype	7	185.98 **	< 0.001
	Error	16	2.95	< 0.001
Stem diameter	Ecotype	7	1.42 **	< 0.001
	Error	16	0.042	< 0.001
Leaf width	Ecotype	7	2.07 **	< 0.001
	Error	16	0.01	< 0.001
Leaf length	Ecotype	7	199.83 **	< 0.001
	Error	16	0.93	< 0.001
Number of garlic	Ecotype	7	28.93 **	< 0.001
	Error	16	2.5	< 0.001
Number of cloves/plant	Ecotype	7	73.27 **	< 0.001
	Error	16	2.08	< 0.001
Bulb diameter	Ecotype	7	564.91 **	< 0.001
	Error	16	8.46	< 0.001
Number of leaves per plant	Ecotype	7	19.17 **	< 0.001
	Error	16	0.38	< 0.001
Dry weight bulb	Ecotype	7	386495.97 **	< 0.001
	Error	16	9726.26	< 0.001
Dry weight of one garlic	Ecotype	7	1022.84**	< 0.001
	Error	16	13.18	< 0.001
Fresh weight bulb	Ecotype	7	535335.02 **	< 0.001
	Error	16	15609.39	< 0.001
allicin	Ecotype	7	2.96 **	< 0.001
	Error	16	0.057	< 0.001
alliin	Ecotype	7	1.85 **	< 0.001
	Error	16	0.003	< 0.001
TPC	Ecotype	7	185.98 **	< 0.001
	Error	16	2.95	< 0.001
TFC	Ecotype	7	19.48 **	< 0.001
	Error	16	0.38	< 0.001
TAC	Ecotype	7	2.07 **	< 0.001
	Error	16	0.01	< 0.001

Ns = non significant * = significant at 5% ** = significant at 1%

Table 5. Means comparison of fresh and dry weight of eight studied Iranian garlic ecotypes in Darab region

Ecotypes	Fresh weight bulb (g/m^2)	Dry weight (g/m^2)	Dry weight of one garlic (g/plant)	Dry weight of one cloves (g/bulb)
Darab	1372.3b	1131.6a	79.0a	4.10c
Tarom	1612.7a	1128.6ab	75.5ab	5.80b
Talesh	1215.6bc	933.3b	75.0ab	6.23b
Tafresh	1319.7bc	1002.3ab	71.3b	5.63b
Hamadan	1129.0c	846.3b	47.3b	4.10c
Ramhormoz	620.0d	406.0c	40.0d	1.80d
Kerman	532.2d	338.0c	43.6cd	3.53c
Bashagard	530.6d	282.3c	33.0e	10.33a
LSD 5%	216.25	170.71	6.284	1.062

The means which have at least one common letter do not have significant difference based on LSD test at 5% level

Table 6. The alliin and allicin content of eight Iranian garlic ecotypes grown in Darab region

Ecotypes	allicin (g/mg dry matter)	alliin (g/mg dry matter)	TPC (mg GAE/g dry matter)	TFC (mg QE/g dry weight)
Darab	4.98 e	3.56 e	12.52 e	1.11 f
Tarom	4.2 f	4.07 d	11.35 f*	1.30 e
Talesh	3.69 g	2.54 f	8.93 g	0.94 g
Tafresh	5.24 e	2.5 h	11.05 f	1.27 e
Hamadan	6.48 b	5.38 a	19.81 b	1.70 c
Ramhormoz	5.82 c	3.12 g	15.69 d	1.46 d
Kerman	5.53 cd	4.49 b	20.63 a	1.84 b
Bashagard	7.12 a	4.21 c	17.42 c	2.12 a
LSD 5%	0.419	0.099	0.329	0.077

The means which have at least one common letter do not have significant difference based on LSD test at 5% level. GAE: Gallic acid equivalent, QE: quercetin equivalents

TPC and TFC

Table 6 showed the ranges of variation of the TPC and TFC contents for the all garlic ecotypes analyzed in the present study. The TPC ranged between 11.05 and 20.63 mg GAL/ g DM and TFC were ranged between 0.94 and 2.12 mg QE/g DM. The highest TPC was obtained in Kerman and Hamedan ecotypes (20.63 and 19.81 mg GAL/g DM, respectively) and the lowest (8.93 mg GAL/ g DM) was observed in Talesh ecotype (Table 6). The Bashagard ecotype showed the highest TFC (2.12 QE/g DM) as compared to other ecotypes. The lowest TFC was obtained in Talesh and Tafresh ecotypes (0.94 and 1.27 QE/g DM, respectively). TPC variation in garlic ecotypes is depended on genetic, agronomic, and environmental factors.

TAC

The results of TAC for eight garlic ecotypes are presented in Table 7. To achieve a more understanding of TAC of the extracts, the FRAP assay was used. As shown in Table 7, Kerman and Talesh had the highest and lowest TAC obtained from methanolic extract, respectively. Aqueous extract of Hamedan ecotype recorded as the greatest content of TAC while, the lowest antioxidant was recorded in aqueous extract of Talesh and Bashagerd ecotypes. Kerman and Hamedan ecotypes showed the highest TAC (25.35 µmol trolox/g FW and 25.21 µmol trolox/g FW, respectively). The lowest TAC was observed in Talesh and Tafresh ecotype with values of 17.08 µmol trolox/g FW and 16.78 µmol trolox/g FW, respectively. Additionally, the methanolic extraction showed higher TAC compared to other aqueous. High variation and significant differences based on FRAP assays among different garlic cultivars were reported in many studies.

Table 7. Evaluation of TAC of the eight Iranian garlic ecotypes (µmol trolox/g FW)

Ecotypes	Methanolic extract	Aqueous extract	Mean
Darab	23.36 e	15.0 e	19.18 C
Tarom	22.10 f*	17.8 c	19.95 B
Talesh	19.77 h	14.4 e	17.08 D
Tafresh	20.37 g	13.2 f	16.78 D
Hamadan	27.82 b	22.6 a	25.21 A
Ramhormoz	24.74 d	16.1 d	20.42 B
Kerman	31.40 a	19.3 b	25.35 A
Bashagard	25.45 c	12.4 f	18.82 C
LSD 5%	0.270	0.978	0.482
Mean	24.37 A	16.35 B	

The means which have at least one common letter do not have significant difference based on LSD test at 5% level

Correlation between characters

Simple correlations amongst the traits are presented in Table 8. There was a moderate to high positive correlation among all vegetative traits except for the number of cloves/bulb and dry weight of one clove. The correlation between number of cloves/plant and dry weight of one cloves was negative and relatively high (-0.82). There was a significant correlation among some biochemical traits (Table 8). The highest significant correlation among the phytochemical characteristics ($r^2 = 0.96$) was observed between methanolic extract and total phenol. The correlation between vegetative and phytochemical characteristics was positively low (not significant) or negative. The highest significant negative correlation ($r^2 = -0.89$) was observed between flavonoids and dry weight of one garlic. There was a significant correlation between flavonoids and bulb diameter ($r^2 = 0.88$). The correlation between latitude and studied traits was calculated (Table 8). The latitude had a significant negative correlation ($r^2 = -0.8$) with methanolic extract and a significant positive correlation ($r^2 = 0.72$) with number of garlic. The garlic ecotypes selected from higher latitudes had more number of garlic and fewer content of methanolic extract.

Table 8. Phenotypic correlation coefficients among traits estimated for eight Iranian garlic ecotypes

Trait	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves per plant	Stem diameter (mm)	Fresh weight of bulb (g/m2)	Dry weight (g/m2)	Bulb diameter (mm)	Dry weight of one garlic (g/plant)	Number of cloves/plant	Dry weight of one cloves (g/plant)	Number of garlic (m2)	Alicin	Alliin	Flavonoids	Phenol	Metanolic	Aqueous	Latitude
Plant height (cm)																			
Leaf length (cm)	0.70*																		
Leaf width (cm)	0.68	0.98**																	
Number of leaves per plant	0.72*	0.89**	0.92**																
Stem diameter (mm)	0.43	0.92**	0.93**	0.82**															
Fresh weight bulb	0.68	0.93**	0.95**	0.97**	0.85**														
dry weight swashing	0.59	0.93**	0.97**	0.95**	0.90**	0.99**													
Bulb diameter (mm)	0.61	0.88**	0.93**	0.91**	0.82**	0.92**	0.95**												
Dry weight of one garlic (g/plant)	0.55	0.81*	0.90**	0.88**	0.83**	0.89**	0.92**	0.91**											
Number of cloves/ plant	-0.25	0.13	0.25	0.30	0.25	0.34	0.43	0.50	0.49										
Dry weight of one cloves (g/plant)	0.36	0.12	0.06	0.03	0.11	-0.02	-0.09	-0.17	-0.07	-0.82**									
Number of garlic (m2)	0.30	0.55	0.59	0.76	0.52	0.72	0.74	0.79	0.62	0.68	-0.45								
Allicin	-0.54	-0.49	-0.61	-0.73*	-0.48	-0.66	-0.66	-0.72	-0.83	-0.50	0.20	-0.58							
Alliin	-0.18	0.02	-0.14	-0.22	-0.04	-0.16	-0.20	-0.37	-0.47	-0.42	0.03	-0.28	0.60						
Flavonoids	-0.41	-0.59	-0.71	-0.77*	-0.61	-0.74*	-0.79*	-0.88**	-0.89**	-0.71*	0.31	-0.78**	0.87**	0.69					
Phenol	-0.62	-0.55	-0.67	-0.75	-0.52	-0.70*	-0.70*	-0.77*	-0.83	-0.33	-0.16	-0.55	0.78*	0.79*	0.85**				
Metanolic	-0.63	-0.54	-0.62	-0.69	-0.47	-0.65	-0.64	-0.73*	-0.71*	-0.20	-0.26	-0.53	0.60	0.74*	0.75*	0.96**			
Aqueous	-0.11	0.09	-0.01	0.03	0.01	0.03	0.02	-0.03	-0.20	0.13	-0.54	0.24	0.11	0.72	0.20	0.57	0.62		
Latitude	0.55	0.57	0.56	0.65	0.44	0.64	0.61	0.67	0.46	0.13	0.16	0.72*	0.08	-0.35	-0.54	-0.65	-0.80**	-0.20	

* and ** indicate statistical significance at $p \leq 0.05$ and $p \leq 0.01$, respectively

Cluster analysis

Based on the results of cluster analysis via the WARD at least variance, the ecotypes were divided into three distinct groups (Fig. 4). Cluster analysis classified Darab, Tafresh, Talesh, Tarom and Ramhormoz ecotypes into group 1. Hamadan and Kerman ecotypes were classified into the second group and Bashagard ecotype classified into the third group. Cluster analysis of the data obtained from the studied traits showed that Talesh and Hamedan ecotypes with the similarity coefficient of 0.34 had the least similarity and Darab and Tafresh ecotypes with the similarity coefficient of 0.97 had the most similarity.

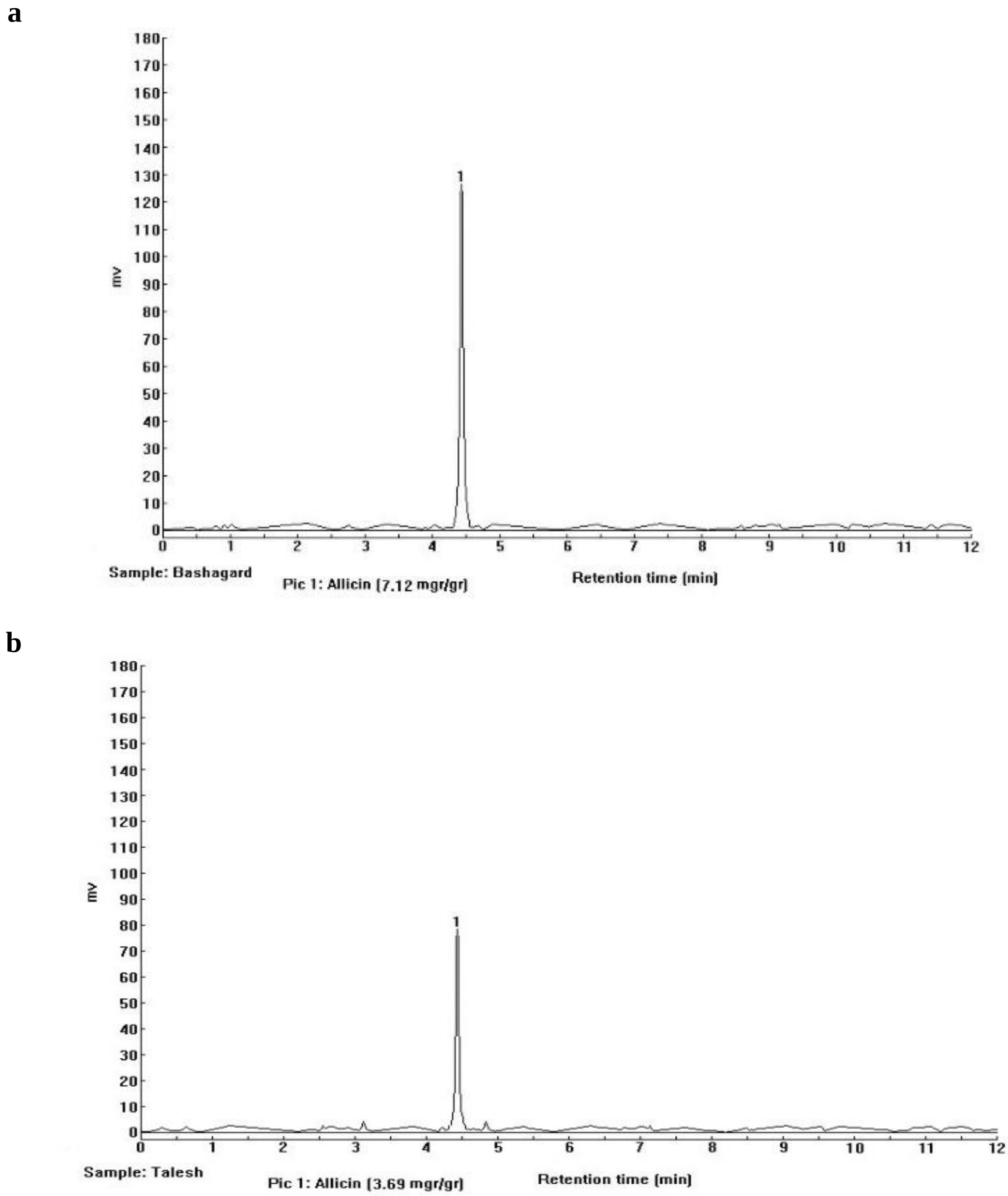

Fig. 3. HPLC chromatograms of allicin content from garlic of Bashagerd (a) and Talesh (b)

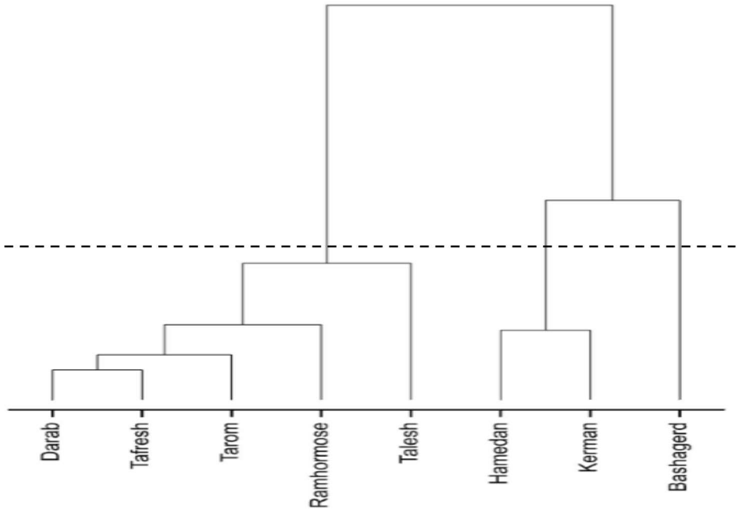

Fig. 4. Dendrogram generated by cluster analysis using vegetative, reproductive and biochemical traits evaluated in eight garlic ecotypes

Discussion

The difference among garlic ecotypes was significant for all vegetative traits indicating a high variety among them. A large variation in morphological traits of garlic ecotypes was reported in many studies (e.g., Panthee et al., 2006; Stavělíková, 2008) that was consistent with the results obtained in this study. In another study, the Egyptian garlic had a more vegetative growth compared to Chinese cultivars (Al-Otayk, 2008). Pooler and Simon (1993) showed that the qualitative and quantitative characteristics of garlic directly depend on the plant arrangement in the field, which in turn depends on the genotype (G), environment (E), and interaction of these two factors (G*E). Different garlic ecotypes showed a great diversity for morphological traits including leaf size, plant height, bulb size, color and shape. The differences between the results of the researchers are attributed to the high variation among the garlic groups as well as the different climatic effects of the experimental site conditions, especially temperature and daytime (Jenderek and Hannan, 2004; Kamenetsky et al., 2004; Simon and Jenderek, 2010)

Significant differences in vegetative and reproductive characteristics among different garlic ecotypes have been reported by other researchers (Figliuolo et al., 2001; Gvozdanovic-Varga et al., 2002; Zahedi et al., 2007; Sandhu et al., 2015). In the study of Abasifar and Dashti (2015), the Hamadan clone showed the highest weight of bulb and cloves among the Iranian garlic ecotypes. The highest number of clove in the bulb and the highest dry matter were observed in the Ahwaz garlic clone. The highest number of leaves, stem diameter and leaf width were observed in Tafresh (Abasifar and Dashti, 2015). The variation in garlic bulb weight can be due to the genetic diversity and the ability of the bulb to adaptation of environmental condition, especially carbon dioxide, light, water and nutrition (Abdel-Razzak and El-Sharkawy, 2012).

Phytochemical characteristic of plants depends on the genotype, environment, and developmental stage of the plants (Hassanzadeh et al., 2017; Hosseini et al., 2018, 2019). Alliin is one of the main bioactive compounds produced in garlic bulb, which is converted to allicin when the bulb is cut or crumpled (Sterling and Eagling, 2001). The selection of garlic clones with the appropriate of allicin content and desirable agronomic traits is essential for the cultivation and production of pharmaceuticals. Allicin content is one of the important factors in determining the quality of different types of commercial garlic, because it directly correlates with the freshness and proper maintenance techniques (Prati et al., 2014). The content of phytochemical compound such as allicin in garlic is greatly variable. It has been known that the amount of allicin, from different regions, is very variable. Additionally agronomic practice and parameters also cause variations in phytochemical compounds (Iberl et al., 1990). Variability in allicin content of garlic collected from different regions could be affected by different factors such as genetic, geographical and environmental factors, nutritional status, agronomy practices and analytical methods (Baghalian et al., 2006; Carolina et al., 2010; Montaño et al., 2011; Khar et al., 2011). Montaño et al. 2011 noted that garlic samples collected from different regions showed a wide variation in the amount of alliin. Sterling and Eagling, (2001) demonstrated that genotype and environmental factors are influenced on allicin yield in Australian grown garlic. The difference in allicin and alliin content of garlic ecotypes in this study, grown in the same environmental conditions and crop management, probably represents a significant genetic variation for these traits. Variation in allicin, allyl methyl thiosulfinate, and allyl trans-1-propenyl thiosulfinate has also been reported in 93 garlic ecotypes (Kamenetsky, 2007).

In a research total polyphenol content (TPC) of garlic was influenced by the geographical location and cultivar (Montaño et al., 2011). According to the report by Gorinstein et al. (2005) total polyphenol content in Polish garlic was greater than the Ukrainian and Israeli garlic cultivars.

In another study genotype and geographical location have been found to influence the total phenolic content and flavonoid content in *Allium* specious. Also, in onions, significant changes in total flavonols between growing seasons have been informed (Rodrigues et al., 2011).

Genetic variation in different garlic ecotype is the primary factor that determines this difference (Waterer and Schmitz, 1994; Beato et al., 2011). Yang et al. (2004) noted that the genotype and location of growth affect the TPC and TFC of Allium species. Genetic, agronomic practice and ecological parameters have main roles in the phenolic composition of crops and it is recognized that cultivar is the main factor that regulates this variation (Chen et al., 2013).

Chen et al. (2013) evaluated the polyphenolic content and antioxidant properties of 43 garlic cultivars in China and reported that the strongest and lowest antioxidant capacity (FRAP, DPPH, and CUPRAC) was obtained in 'Hanzhong purple' and 'Gailiang' cultivars respectively. Saxena et al. (2007) reported that the contents of bioactive compounds and related antioxidant activity in vegetables are influenced by many factors such as geographical region, climatic and storing conditions, and degree of ripeness.

Significant correlations between the content of polyphenols and antioxidant activity in garlic have been reported in several previous studies (Baghalian et al., 2005; Chekki et al., 2014; Abasifar and Dashti, 2015).

In the study of Fakhrfeshani and Shahriari, (2013), Hamedan and Ali Abad Katoul (Golestan) ecotypes had the least similarity. In this study, the ecotypes of Kerman and Hamedan provinces were included in one group. Based on the results of this study, the diversity of traits and geographical origin do not follow a similar and significant pattern because ecotypes of the same geographical origin are located in different groups or vice versa, and ecotypes of different geographic origins were grouped into one group. Similar results in agreement with this study have been reported by Bahador et al. (2014).

In a research by Baghalian et al. (2005) 24 Iranian garlic ecotypes categorized by cluster analysis in 6 main groups (A, B, C, D and E) whereas ecotype 20 and 22 were presented in cluster groups E and F, respectively.

Conclusion

In this study, the adaptation and phytochemical content (alliin and allicin, TAC, TPC, TFC) in eight Iranian garlic ecotypes in the Darab region were evaluated. The results of this study indicated that the studied ecotypes showed great diversity in terms of morphological and phytochemical characteristics due to their genetic diversity. The phenotypic growth of garlic was more announced in Tarom and Tafresh ecotypes, but in terms of phytochemical properties (allicin content, alliin content, TPC, TFC, and TAC) that are important features and have a great impact on the shelf life and nutritional aspects of garlic, Hamedan and Kerman ecotypes had the best performance altogether. The dry weight of one garlic (g/plant) is another important parameter in the garlic production that Darab, Tarom, and Talesh ecotypes showed the highest values. In garlic cultivation, the dry weight of the one garlic and their number per plant is of great importance. For this goal, Tarom and Talesh can be the best choices. Where the goal is to use the medicinal properties of the plant, Hamedan and Kerman ecotypes can be used to achieve a greater concentration of the desired ingredients.

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Conflict of interest

The authors indicate no conflict of interest for this work.

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