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Research Article

Phenological, morpho-physiological, and biochemical attributes of barberry (*Berberis integerrima* L.) in different habitats of Iran

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Abstract

Berberis integerrima L. belongs to the Berberidaceae family and is one of the small fruits with many medicinal properties, which is considered among the horticultural products with high plant diversity and favorable genetic reserves. This research aimed to study barberry's phenological, morpho-physiological, and biochemical attributes in five different habitats in Kerman province, Iran (Bam, Jiroft, Anbarabad, Raber, and Baft) during 2019-2020. The results of the phenological traits showed that the Jiroft and Anbarabad habitats have the maximum number of days until the end of vegetative growth, the number of days from activation of buds to the beginning of fruit formation, the number of days until the physiological maturity of the fruit, the number of days from activation of buds to fruit coloring, and the lowest number of days until flowering. The mean comparison showed that in terms of yield, the investigated habitats from the highest production to the lowest included Anbarabad> Baft> Bam> Rabar> Jiroft. The highest average traits of panicle length (43.38 cm), number of fruits per panicle (26.6), 1000-fruit weight (141.9 g), 1000-seedless fruit weight (128.4 g), seed length (1.16 mm), seed diameter (0.97 mm), fruit length and diameter (19.03 and 5.41 mm), yield (16.69 kg per stem), and the number of thorns 10 cm (13.5 pieces) was observed in Anbarabad habitat. The yield was negatively and significantly correlated with fruit length, titratable acidity, anthocyanin, DPPH, and phenol. Interestingly, soil nitrogen, phosphorus, and potassium had a positive effect on increasing the barberry yield. On the other hand, increasing the longitude, latitude, and altitude decreases yield. Anbarabad and Jiroft habitats had the highest and lowest averages of pH, titratable acidity, total soluble solids, chlorophyll, and carotenoid content, respectively. Generally, the results of this study indicate a high genetic diversity in phenological, morphological, chemical, and physiological aspects in different habitats. Moreover, the results showed that Anbarabad was significantly superior in phenological, morphological, yield, and phytochemical traits compared to other ecotypes.

Keywords: anthocyanin; latitude; longitude; small fruits; titratable acidity; yield

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Introduction

Barberries are a broad class of spiny evergreen or deciduous shrubs belonging to the Berberidaceae family (Alemardan *et al*., 2013). They are important due to their different parts' nutritional and medicinal properties and their ornamental applications. Genus Berberis, the most well-known genus in Berberidaceae, includes more than 12 genera and 660 species that have been identified around temperate climates of the northern hemisphere (Khodabandeh *et al*., 2022). *B. crataegina*, *B. khorasanica*, *B. orthobotrys*, *B. vulgaris*, and *B. thunbergii* var. *atropurpurea* are found in Iran (Zarei *et al*., 2015). Seedy barberry (*B. integrrima* L.) belongs to the Berberidaceae family and has small fruits with nutritional and medicinal values (Abudureheman *et al*., 2022). This plant grows in Asia and Europe and has been used extensively as a medicinal plant in traditional medicine. In Iranian traditional medicine, several properties, such as antibacterial, antipyretic, antipruritic, and antiarrhythmic, have been reported with unknown mechanisms of action. Incredible structural diversity among barberries' active components makes them a valuable source of novel therapeutic compounds (Khodabandeh *et al*., 2022). The fruit growth of this type of barberry is completed in September, it has yellow flowers, and its black color is completed in autumn (Sarraf *et al*., 2019). This fruit has two to three small seeds, and the fruit is very juicy, and contains a lot of anthocyanin pigment and antioxidant compounds. The amount of anthocyanin in the plant largely depends on the harvest time and environmental factors (Alemardan *et al*., 2013). The plants grow naturally in semi-arid and humid highlands, and their geographical distribution in Iran is mainly in the north and northeast regions of the country, as well as the highlands of Azerbaijan and Kerman (Tavakoli Kaghaz *et al*., 2021). Based on the available sources, different species of barberry are scattered in many mountainous regions of Iran, and it is necessary to investigate their morpho-physiological, phenological, and biochemical characteristics (Alavi and Mazloumzadeh 2012; Alemardan *et al*., 2013).

When the plant is affected by different ecological conditions, it changes the quantity and quality of its secondary metabolites to adapt to these conditions (Pant *et al*., 2021). Therefore, the medicinal plant cultivars that have grown in different ecological conditions form different and diverse types in terms of the quantity and quality of effective substances, which, of course, leads to differences in the scope of their therapeutic and biological activity (Li *et al*., 2020). The genetic flexibility of plant populations, which has made the occurrence of morphological and physiological variations possible, gradually, under the influence of the force of evolution, in different geographical areas, creates populations of the same species that, in terms of physiological, chemically, and botanically characteristics and finally genetically they are different from each other (Li *et al*., 2020; Pant *et al*., 2021). Due to the changes in climatic conditions, the appearance and morphology of the plant change. These changes further cause changes in the production of secondary metabolites and create different chemical types (Waterman and Mole 2019).

The growing trend of consumption of medicinal plants and the production of herbal medicines without the development of suitable methods of planting and harvesting destroys valuable species in nature. Therefore, the cultivation of medicinal plants at agricultural levels and their industrial processing by relevant specialists are necessary after breeding studies (Nwafor *et al*., 2021; Taghouti *et al*., 2022). Among the most important environmental factors affecting the growth of medicinal plants, which have a significant impact on the quantity and quality of their effective substances, we can mention light, temperature, rainfall, day length, latitude, soil characteristics, altitude, and nutrition (Askari-Khorasgani and Pessarakli 2019). There have been studies about the quantitative and qualitative of plants in different habitats. For example, *Stachys pilifera* L. (Heydari *et al*., 2019), *Thymus migricus* L. (Yavari *et al*., 2010) and barberry (Jannatizadeh and Khadivi-Khub, 2016; Khromykh *et al*., 2018; Goodarzi *et al*., 2018; Rezaei *et al*., 2020).

Seedy barberry trees grown in different habitats have different morphological, phenological, cytogenetic, and phytochemical characteristics. Considering the importance of barberry in Iran, the present study was conducted to investigate the morphological, yield, and physiological diversity to assess the feasibility of domestication of seedy barberry.

Materials and Methods

Experimental conditions and plant materials

To study the phenological, morphological, phytochemical, and physiological characteristics of barberry in Kerman province, five different habitats were selected in the cities of Jiroft (Sarbijan), Anbarabad (Jabalbarz), Raber (Bandar Hanza), Baft (Goghar) and Bam (Dehbakri). The number of 10 trees (same size and age) with a distance of 10 m in each habitat was randomly selected. The geographical characteristics of the studied plants are shown in Table 1. Also, the average monthly temperature and rainfall related to the growing season in different habitats are presented in Table 2. The statistics of temperature and rainfall in the studied habitats showed that in terms of the annual average temperature, from the highest to the lowest, the habitats were located in the order of Bam > Rabar > Jiroft > Baft > Anbarabad, but in terms of the annual average amount of rainfall in the order of Anbarabad > Rabar > Jiroft > Bam > Baft.

Table 1. Geographical and soil characteristics of the studied habitats in Kerman province, Iran

Habitats	Longitude (E)	Latitude (N)	Altitude (m.a.s.l)	Average annual temperature (°C)	Relative humidity (%)	Average annual rainfall (mm)	N (%)	P (mg/kg)	K (mg/kg)
Jiroft	57° 44′ 13″	28° 40′ 13″	720	21.1	43	176.2	0.09	15.4	100.3
Anbarabad	57° 08′ 11″	28° 15′ 02″	601	21.2	41	175.3	0.15	16.3	130.1
Rabor	56° 45′ 19″	29° 27′ 25″	2342	15.6	39	247.1	0.04	9.12	110.8
Baft	56° 37′ 14″	29° 14′ 10″	2280	15.3	39	247.2	0.06	10.2	80.4
Bam	58° 21′ 32″	29° 06′ 26″	1061	23	30	61.4	0.02	13.6	70.3

Table 2. Statistics of rainfall and temperature (annual average) in the five habitats of Kerman province, Iran during 2019-2020

	Jiroft		Anbarabad		Rabor		Baft		Bam	
	Rainfall (mm)	Temp. (°C)	Rainfall (mm)	Temp. (°C)	Rainfall (mm)	Temp. (°C)	Rainfall (mm)	Temp. (°C)	Rainfall (mm)	Temp. (°C)
Avr.	2.65	25.11	13.1	16.10	5.735	27.41	0.825	19.77	2.15	28.02
Max	10.8	35.94	42.8	26.79	27.5	38.39	5	30.21	7	38.21
Min	0	12.87	0	4.96	0	14.69	0	7.41	0	15.41

Measurement of phenology attributes

To investigate phenology attributes, the plants were visited once a week from the end of February until the leaves fall (November) during 2019-2020. The time of bud activation (the beginning of vegetative growth), the end of vegetative growth (time fall of leaves), the beginning of flowering up to 25, 50, and 100% of flowering (equal to the length of the flowering period), the number of days from the activation of buds to the beginning of fruit formation, the number of days from the activation of buds to fruit coloring, the number of days until the physiological maturity of the fruit (when the size of the fruit does not increase) and the number of days until physiological harvest were recorded for each habitat separately.

Measurement of morphological and yield characteristics

The investigated morphological and yield characteristics include panicle width and length, number of fruits per panicle, 1000-fruit weight, 1000-seedless fruit weight, 1000-seed weight, seed length and diameter, fruit length and diameter, fruit length to diameter ratio, fruit flesh diameter, product yield, and the number of thorns in 10 cm. Meters, rulers, calipers, scales, and counting were used to measure the mentioned traits (Alemardan *et al*., 2013; Jannatizadeh and Khadivi-Khub, 2016).

Measurement of phytochemical and physiological characteristics

The leaves and fruits of the selected trees in each habitat were sampled and then analyzed. The fruits were collected from the plant when they ripened on the 12th of October, and the leaves were harvested on the 6th of September. A mortar was used to extract barberry extract. First, the seed was separated from the fruit, then the fruit without seeds was turned into a relatively smooth paste in the mortar. The crushed seeds were poured into a cloth and put under pressure to prepare smooth fruit juice. In this way, the liquid passed through the fabric and became smooth. Then, to obtain the fully filtered extract, each sample was passed through filter paper separately (Hassanpour and Alizadeh 2016).

Measurement of fruit pH

JENWAY 3520 pH meter was used to determine the pH of the extracts. The pH measurement was repeated four times for the accuracy of the test and to reduce the error.

Measurement of fruit titratable acidity (malic acid)

First, 10 mL of strained barberry extract in each repetition was brought to a volume of 100 mL with distilled water, then two drops of phenolphthalein were added to 10 mL of this diluted extract, and the titer was 0.1 N. Considering that the reagent used is phenolphthalein, which should change from colorless to purple at pH 7.3, and since it is similar in color to crimson, the color change was not quickly evident. Therefore, a pH meter was used. This way, the pH meter electrode was placed inside the sample, and 0.1 N soda solution was added drop by drop and stirred until the pH reached 8.3. After the end of the process, the consumption of soda was noted, and the percentage of titratable acidity in terms of malic acid was calculated by the following method (Eq. 1) (Ardestani *et al*., 2013):

Eq. 1: Titratable acidity (%) = (mEq × N of soda x consumption of soda (ml) × 100) / sample weight

One mL of 0.1 N soda is equivalent to 0.0067 g of malic acid.

Measurement of fruit total soluble solids (TSS) or Brix

A handheld refractometer (ERMA, Japan) was used to measure TSS. For this purpose, two drops of fruit juice, after straining, were placed on the refractometer measuring plate, and the resulting number was read.

Measurement of anthocyanin content

With modifications, the anthocyanin content was measured spectrophotometrically, as described previously (Sakamoto and Suzuki, 2019). Fresh samples were dried in an oven at 55 °C for six h. Dried samples were weighed and soaked in 1 mL methanol containing 1% HCl and were incubated at 95 °C for 15 min. The sample was then cooled to room temperature. After removing the solids, the absorbance of the supernatant was measured at 533 nm, and a standard calibration curve was prepared using cyanidin-3-glucoside (CY) (Farrokhi *et al*., 2021).

Measurement of fruit antioxidant properties

The antioxidant capacity of barberry extract was determined through DPPH (Brand-Williams *et al*., 1995). For this purpose, 0.2 g of each fruit sample was ground in a mortar with the help of liquid nitrogen, and 10 mL of methanol was added to it. After a little stirring, it was poured into small beakers and kept at room temperature for one hour. The extracts were filtered with filter paper. Then they were centrifuged at 3000 rpm for 5 min. 50 µL of methanolic extract was added to 950 µL of DPPH solution. After adding DPPH, the mixture was immediately stirred and then kept at room temperature for 15 min in the dark until the solution reached a constant state. The decrease in absorbance was determined at the wavelength of 515 nm. This experiment was repeated three times for each treatment. Then, the antioxidant capacity of the extracts was calculated as DPPH inhibition percentage using Eq. 2.

Eq. 2: DPPH (%) = (Acont – Asamp) × 100/Acont

Where DPPH: inhibition percentage, Asamp: absorption rate (sample + DPPH, Acont: DPPH absorption rate.

Measurement of fruit total phenol

The total phenol content of the fruit was measured using the Folin-Siocalcho method (Ainsworth and Gillespie, 2007). For this purpose, one g of the sample was ground in a mortar with the help of liquid nitrogen. Then 10 mL of pure methanol was added to extract phenolic compounds. Then the extracts were filtered with filter paper. To prepare the standard solution, first, a stock solution of gallic acid (0.1 g of gallic acid with pure methanol to a volume of 100 mL), Folin (5 mL of Folin with distilled water to a volume of 50 mL), and 7.5% sodium carbonate (adding 5 (1 g of sodium carbonate in 20 mL of distilled water) was prepared. The procedure was as follows: 10, 15, 20, 25, and 50 µL of gallic acid were poured into small glass containers, and 2500 µL of folinic acid and 2000 µL of sodium carbonate were added to each glass. The absorbance of the solutions was read at a wavelength of 760 nm.

Measurement of leaf chlorophyll and carotenoids

First, 0.4 g of leaf tissue from each replicate was ground with the help of liquid nitrogen in a mortar, and 5 mL of 80% acetone (20% distilled water: 80% acetone) was added to it. The obtained mixture was centrifuged at 5000 rpm for 15 min. Finally, the clear acetone extract was separated, and its volume reached 5 mL (initial volume) with pure acetone. Chlorophyll and carotenoids were measured using the spectrophotometric method at 646.2, 663.2 and 470 nm wavelengths. The following formulas were used to determine the amount of total chlorophyll and carotenoids, respectively (Lichtenthaler and Wellburn, 1983):

Eq. 3: Chlorophyll-a (mg/g FW) = 12.25 A663.2- 2.79 A646.8

Eq. 4: Chlorophyll-b (mg/g FW) =21.50 A646.8 -5.10 A663.2

Eq. 5: Total chlorophyll (mg/g FW) = Chlorophyll-a + Chlorophyll-b

Eq. 6: Carotenoid (mg/g FW) = (1000 × A470-1.8 Chl-a-85.02 Chl-b)/198

Statistical analysis

After checking the data distribution normality assumption through employing Kolmogorov-Smirnov and Shapiro-Wilk tests, data were analyzed using a Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.4) and Minitab version 19. Statistical analysis was performed using a completely randomized design (CRD), and means were compared with a Least Significant Difference (LSD) test at $p \leq 0.05$. The p-values of less than 0.05 were considered statistically significant. Simple correlation (Pearson) between traits, as well as cluster analysis between the studied habitats, were performed using Microsoft Excel (version 2013) and Minitab (version 19) software.

Results

Phenological attributes

Based on results, Jiroft habitat had the earliest onset of vegetative growth (bud activation), and Bam habitat had the latest start of vegetative growth. On the contrary, the vegetative growth period was 90 days in Jiroft, but 44.5 days in Bam. The minimum and maximum length of vegetative growth period were obtained in Baft and Anbarabad habitats (37.5 and 92 days), respectively. Contrary to these results, the flowering period was the lowest in Anbarabad (31.0 days along with Jiroft, which was 30.5 days) and the highest average (38.5 days) in Baft. The shortest length of the growth period (activation of buds until harvest maturity) was related to Baft and Raber (164.0 and 169.5 days, respectively), and the longest was in Jiroft and Anbarabad habitats (215 and 218 days, respectively) (Table 3).

Table 3. Average phenological characteristics of barberry (*Berberis integerrima* L.) plant in different habitats of Kerman province, Iran

Habitats	Jiroft	Anbarabad	Rabor	Baft	Bam
Beginning of vegetative growth	5-Mar	9-Mar	25-Mar	29-Mar	31-Mar
Vegetative growth period (day)	90	92	42	37.5	44.5
Beginning of flowering up to 25% of flowering (days)	9	8.5	13.5	12.5	13.5
Beginning of flowering up to 50% of flowering (days)	15.5	16.5	22.5	20.5	22
Beginning of flowering up to 100% of flowering (days)	30.5	31	37	38.5	37.5
The number of days from activation of buds to the beginning of fruit formation	120.5	123	79	76	82
The number of days from activation of buds to fruit coloring	183	189	138	132	142
The number of days until the physiological maturity of the fruit	200.5	204.5	153.5	150	161.5
The number of days until harvest maturity	215	218	169.5	164	170.5

Morphological traits, yield, and yield components

The obtained results showed that there is a significant difference ($p<0.01$) in terms of morphological and yield traits, including panicle width and length, number of fruits per panicle, 1000-fruit weight, 1000-seedless fruit weight, 1000-seed weight, seed length and diameter, fruit length and diameter, fruit length to diameter ratio, fruit flesh diameter, product yield, and the number of thorns in 10 cm were found among the studied habitats. The highest average traits of panicle length (43.38 cm), number of fruits per panicle (26.6), 1000-fruit weight (141.9 g), 1000-seedless fruit weight (128.4 g), seed length (1.16 mm), seed diameter (0.97 mm), fruit length and diameter (19.03 and 5.41 mm), yield (16.69 kg per stem), and the number of thorns 10 cm (13.5 pieces) was observed in Anbarabad habitat. On the other hand, in Jiroft habitat, the average yield and yield components such as panicle width and length, number of fruits per panicle, 1000-fruit weight, 1000 seedless fruit weight, seed length and diameter, fruit length and diameter, fruit flesh diameter, yield, and the number of thorns in 10 cm had the lowest value among the studied habitats. The average mean comparison showed that in terms of product yield (economic organ of barberry), the investigated habitats from the highest production to the lowest production included Anbarabad> Baft> Bam> Rabar> Jiroft (Table 4).

Table 4. Mean comparison of the yield attributes of barberry (*Berberis integerrima* L.) plant in different habitats of Kerman province, Iran

Habitat	Panicle width (cm)	Panicle length (cm)	Number of fruits per panicle	1000-fruit weight (g)	1000-seedless fruit weight (g)	1000-seed weight (g)	Seed length (mm)
Jiroft	3.82±2.42 c	29.42±2.68 d	21.6±4.2 b	120.83±8.01 b	107.49±7.76 b	13.34±0.58 a	0.95±0.08 c
Anbarabad	3.68±1.68 c	43.38±1.88 a	26.6±2.59 a	141.96±8.79 a	128.47±9.23 a	13.5±0.68 a	1.16±0.11 a
Rabor	5.83±2.63 ab	35.63±2.99 bc	25.8±3.39 a	122.33±8.01 b	108.69±7.04 b	13.64±1.6 a	1.03±0.09 bc
Baft	7.41±1.82 a	37.41±1.82 b	21.5±3.6 b	119.17±9.42 b	106.05±6.91 b	13.12±2.59 a	1.01±0.1 bc
Bam	4.16±1.96 bc	33.96±2.19 c	26.5±3.63 a	122.23±7.9 b	108.52±8.01 b	13.71±0.47 a	1.08±0.05 b
LSD =0.05%	1.92	2.12	3.17	7.60	7.05	1.29	0.081
Habitat	Seed diameter (mm)	Fruit length (mm)	Fruit diameter (mm)	Fruit length to diameter ratio	Fruit flesh diameter (mm)	Yield (kg per stem)	The number of thorns in 10 cm
Jiroft	0.79±0.06 c	14.09±1.12 d	3.42±0.9 c	4.41±1.33 a	0.65±0.15 c	12±1.47 c	10.3±1.95 b
Anbarabad	0.97±0.09 a	19.03±1.38 a	5.41±0.69 a	3.56±0.45 b	0.81±0.12 ab	16.69±1.32 a	13.5±2.55 a
Rabor	0.86±0.08 bc	15.43±1.63 bc	4.35±0.89 b	3.67±0.83 ab	0.73±0.15 bc	13.07±1.82 bc	14.2±2.25 a
Baft	0.81±0.09 c	14.71±1.3 cd	5.78±0.9 a	2.64±0.69 c	0.88±0.15 a	14.41±1.57 b	12.6±2.46 a
Bam	0.9±0.09 b	16.35±1.07 b	4.36±0.7 b	3.87±0.86 ab	0.73±0.12 bc	13.44±1.46 b	12.4±2.63 ab
LSD =0.05%	0.075	1.18	0.73	0.79	0.12	1.38	2.14

Means (±SD) followed by the same letter in each column are not significantly different according to LSD test at 5 % level

Phytochemical and physiological characteristics

Based on the obtained results, there was a statistically significant difference between habitats in pH, TSS, fruit anthocyanin content, fruit antioxidant activity, fruit phenol content, and leaf chlorophyll and carotenoid content ($p<0.01$). In terms of fruit titratable acidity, no significant difference was observed between habitats. Results showed that the pH in Anbarabad had the highest value (3.64), which was significantly different from the samples of other habitats. Also, the lowest average of this trait was observed in the samples of four other habitats, namely Jiroft (3.04), Raber (2.59), Baft (2.77), and Bam (3.1). In other words, there was no statistically significant difference between these four habitats regarding fruit pH (Table 5).

The fruit titratable acidity of the fruit varied from 2.9 to 3.35%. From a numerical point of view, the highest and lowest titratable acidity was related to Raber and Bam habitats, respectively (Table 5).

Based on the mean comparison results, the highest TSS was observed in Anbarabad samples with an average of 17.5%, and the lowest TSS was in Jiraft and Baft samples (11.6 and 11.6%, respectively) (Table 5).

As shown in Table 5, the anthocyanin content in the samples of Jiroft (81.8 mg/100 g) and Raber (72.91 mg/100 g) had the highest average compared to the other habitats. Barberry samples in Anbarabad and Bam habitats had the lowest anthocyanin content (50.3 and 52.2 mg/100 g, respectively).

The mean comparison results showed that the samples of Anbarabad habitat with 50.3% of antioxidant activity had the lowest average of this trait. In terms of the antioxidant activity of the samples from four other habitats, Jiraft, Raber, Baft, and Bam had the highest average of this attribute (61.8, 59.4, 55.8, and 52.3%, respectively) (Table 5).

Based on Table 5 results, the highest phenol content was found in Raber and Jiroft samples (12.9 and 12.1 mg of gallic acid per g FW, respectively). The lowest total phenol content was observed in Bam (8.4 mg of gallic acid per g FW).

The leaf chlorophyll content was the highest in Anbarabad samples (0.38 mg/g FW), and the chlorophyll content in the other four habitats of Jiroft, Raber, Baft, and Bam (0.28, 0.25, 0.30, and 0.29 mg/g FW, respectively) had the lowest average (Table 5).

Regarding carotenoid content, Anbarabad samples with an average of 0.19 mg/g FW had the highest and most significant difference with other habitats. The lowest leaves carotenoid content was observed in Jiroft samples with an average of 0.09 mg/g FW (Table 5).

Table 5. Mean comparison of the fruit and leaves phytochemical and physiological characteristics of barberry (*Berberis integerrima* L.) plant in different habitats of Kerman province, Iran

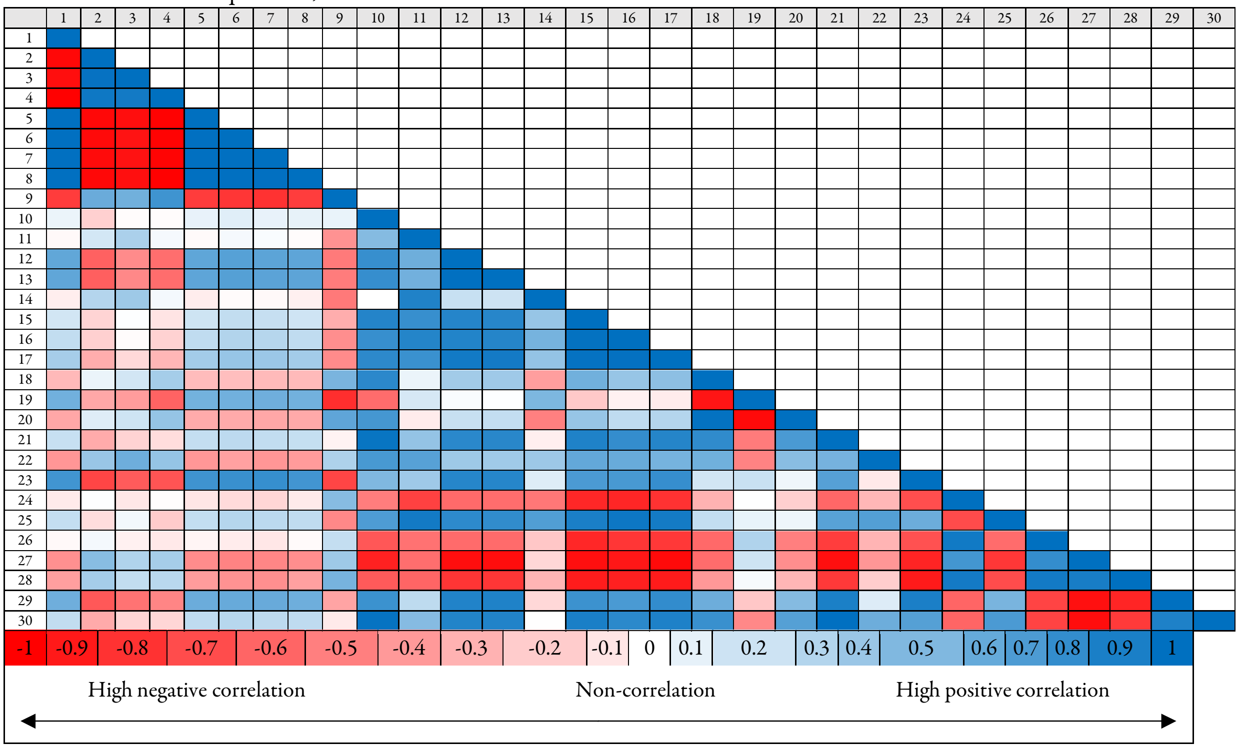
Habitats	pH	Titratable acidity (%)	Total soluble solids (%)	Anthocyanin ($mg.100\ g^{-1}$)	DPPH (%)	Phenol ($mg\ GA.g^{-1}$)	Leaf chlorophyll (mg/g FW)	Leaf carotenoids (mg/g FW)
Jiroft	3.04±0.3 b	3.4±0.37 a	11.6±1.34 c	81.8±5.74 a	61.8±6.54 a	12.1±0.6 ab	0.28±0.02 b	0.09±0.02 c
Anbarabad	3.64±0.3 a	2.96±0.38 a	17.5±0.65 a	50.3±2.26 c	32.2±7.71 b	6.41±1.32 d	0.38±0.07 a	0.19±0.03 a
Rabor	2.59±0.31 b	3.35±0.23 a	15.0±1.35 b	79.92±4.9 a	59.4±1.93 a	12.9±0.48 a	0.25±0.03 b	0.12±0.02bc
Baft	2.77±0.11 b	3.31±0.29 a	11.6±1.52 c	64.8±4.78 b	55.8±5.53 a	11.4±0.85 b	0.3±0.02 b	0.14±0.02 b
Bam	3.1±0.34 b	2.9±0.24 a	14.8±1.35 b	52.2±3.16 c	52.3±6.49 a	8.4±0.26 c	0.29±0.03 b	0.12±010 bc
LSD =0.05%	0.51	0.56	2.32	7.92	10.87	1.46	0.07	0.037

Means (±SD) followed by the same letter in each column are not significantly different according to LSD test at 5 % level

Simple correlation coefficient and cluster analysis

As shown in Table 6, Pearson's correlation coefficient matrix of all the measured variables showed significantly negative and positive correlations between the phenological, morphological, yield, yield components, phytochemical, and physiological attributes of barberry. For example, the yield was significantly and positively correlated with panicle length, 1000-fruit weight, 1000-seedless fruit weight, seed length and diameter, fruit length and diameter, fruit flesh diameter, the number of thorns, pH, TSS, leaf chlorophyll, and leaf carotenoids. However, the yield was negatively and significantly correlated with fruit length, titratable acidity, anthocyanin, DPPH, and phenol.

Table 6. Pearson correlation coefficient between phenological, morphological, yield, yield components, phytochemical, and physiological attributes of barberry (*Berberis integerrima* L.) plant in different habitats of Kerman province, Iran



Legend: 1. Vegetative growth period; 2. Beginning of flowering up to 25% of flowering; 3. Beginning of flowering up to 50% of flowering; 4. Beginning of flowering up to 100% of flowering; 5. The number of days from activation of buds to the beginning of fruit formation; 6. The number of days from activation of buds to fruit coloring; 7. The number of days until the physiological maturity of the fruit; 8. The number of days until harvest maturity; 9. Panicle width; 10. Panicle length; 11. Number of fruit per panicle; 12. 1000-fruit weight; 13. 1000-seedless fruit weight; 14. 1000-seed weight; 15. Seed length; 16. Seed diameter; 17. Fruit length; 18. Fruit diameter; 19. Fruit length to diameter ratio; 20. Fruit flesh diameter; 21. Yield; 22. The number of thorns; 23. pH; 24. Titratable acidity; 25. Total soluble solids; 26. Anthocyanin; 27. DPPH; 28. Phenol; 29. Leaf chlorophyll; 30. Leaf carotenoids

Interestingly, soil nitrogen, phosphorus, and potassium had a positive effect on increasing the barberry yield. On the other hand, increasing the longitude, latitude, and altitude decreases yield. Climatic parameters such as the average annual temperature, relative humidity, and average annual rainfall had no significant negative or positive correlation with yield (Table 7).

Table 7. Pearson correlation coefficient between barberry yield with geographical and soil characteristics of the studied habitats in Kerman province, Iran

	Longitude	Latitude	Altitude	Average annual temperature	Relative humidity	Average annual rainfall	N	P	K
Yield	-0.21*	-0.40**	-0.19ns	0.05ns	0.06ns	0.05ns	0.63**	0.28*	0.46**

ns: non-significant; * and **: significant at 5 and 1% probably level, respectively.

As shown in Table 8 and Figure 1, five habitats were classified into three clusters; the first and second clusters included one habitat (Jiroft and Anbarabad, respectively), and Rabar, Baft, and Bam as the third cluster. The first cluster in terms of fruit biochemical and physiological characteristics such as anthocyanin content, DPPH, and total phenol content, and the second cluster in yield and yield components attributes had the highest coefficients. Finally, the third cluster with the highest habitats had a significant superiority in phenology traits.

Table 8. Cluster analysis and grouping for phenological, morphological, yield, yield components, phytochemical, and physiological attributes of barberry (*Berberis integerrima* L.) plant in different habitats of Kerman province, Iran

Variable	Cluster-1	Cluster-2	Cluster-3	Grand centroid
1	90.000	92.000	41.333	61.200
2	9.000	8.500	13.167	11.400
3	15.500	16.500	21.667	19.400
4	30.500	31.000	37.667	34.900
5	120.500	123.000	79.000	96.100
6	183.000	189.000	137.333	156.800
7	200.500	204.500	155.000	174.000
8	215.000	218.000	168.000	187.400
9	3.817	3.679	5.800	4.979
10	29.417	43.379	35.667	35.959
11	21.600	26.600	24.600	24.400
12	120.827	141.964	121.242	125.304
13	107.485	128.466	107.755	111.843
14	13.342	13.498	13.488	13.461
15	0.951	1.164	1.038	1.046
16	0.788	0.974	0.857	0.866
17	14.093	19.029	15.497	15.922
18	3.420	5.407	4.828	4.662
19	4.408	3.561	3.393	3.630
20	0.654	0.807	0.777	0.759
21	12.003	16.690	13.643	13.924
22	10.300	13.500	13.067	12.600
23	3.041	3.643	2.819	3.028
24	3.400	2.958	3.185	3.183
25	11.690	17.507	13.840	14.143
26	81.857	50.360	65.659	65.839
27	61.823	32.247	55.809	52.299
28	12.147	6.407	10.921	10.263
29	0.282	0.383	0.281	0.302
30	0.093	0.194	0.128	0.134

Legend: 1. Vegetative growth period; 2. Beginning of flowering up to 25% of flowering; 3. Beginning of flowering up to 50% of flowering; 4. Beginning of flowering up to 100% of flowering; 5. The number of days from activation of buds to the beginning of fruit formation; 6. The number of days from activation of buds to fruit coloring; 7. The number of days until the physiological maturity of the fruit; 8. The number of days until harvest maturity; 9. Panicle width; 10. Panicle length; 11. Number of fruit per panicle; 12. 1000-fruit weight; 13. 1000-seedless fruit weight; 14. 1000-seed weight; 15. Seed length; 16. Seed diameter; 17. Fruit length; 18. Fruit diameter; 19. Fruit length to diameter ratio; 20. Fruit flesh diameter; 21. Yield; 22. The number of thorns; 23. pH; 24. Titratable acidity; 25. Total soluble solids; 26. Anthocyanin; 27. DPPH; 28. Phenol; 29. Leaf chlorophyll; 30. Leaf carotenoids

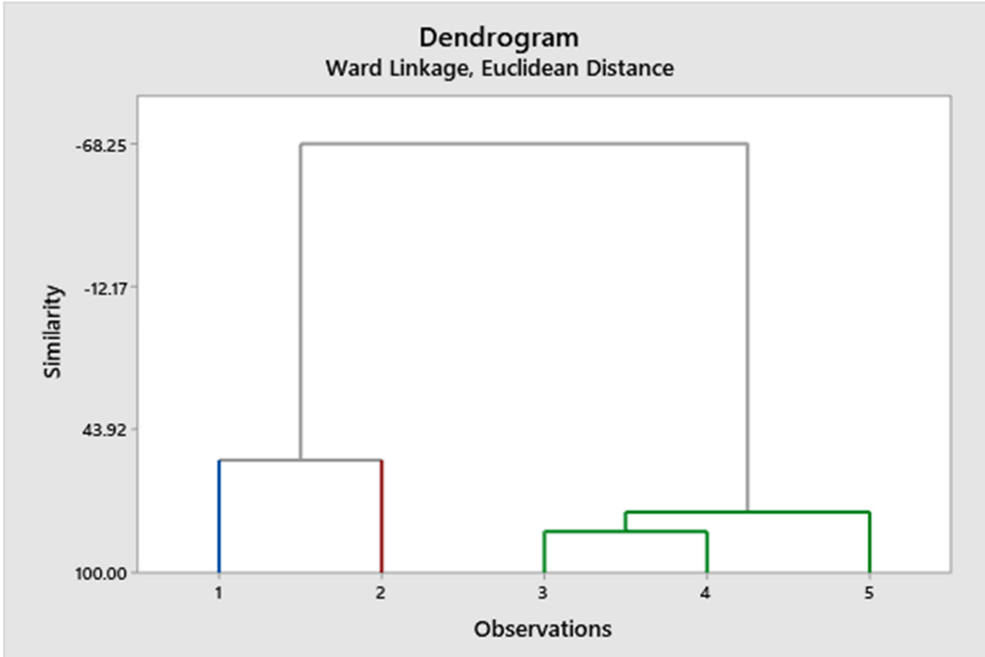


Figure 1. Cluster analysis for phenological, morphological, yield, yield components, phytochemical, and physiological attributes of barberry (*Berberis integerrima* L.) plant in different habitats of Kerman province, Iran
(1: Joroft; 2: Anbarabad; 3. Rabor; 4. Baft; and 5. Bam)

Discussion

The objectives of the current research were to study the phenological, morphological, yield, yield components, phytochemical, and physiological attributes of barberry in different habitats of Kerman province, Iran. The results showed a significant difference in barberry phenological characteristics in different habitats. The beginning of fruit coloring was in early September, and the number of days until the physiological maturity of the fruit was late September. Anbarabad has a higher temperature than other cities due to its lower altitude (601 m.a.s.l). Therefore, these factors increase the length of the vegetative growth period, the number of days from the activation of the buds to the beginning of fruit formation, the number of days from activation of buds to fruit coloring, the number of days until the physiological maturity, and the number of days until harvest maturity, while has been reduced the number of days of flowering to 25, 50 and 100. Zargoosh *et al*. (2019) stated that among the most important environmental factors influential in the growth of medicinal plants, which have a major effect on the growth parameters and the quality of their effective substances, can be light, temperature, rainfall, length of day, latitude, soil characteristics, altitude, and nutrition. Farrokhi *et al*. (2021) reported that warm and sunny regions produce more flowers and increase vegetative growth. These researchers listed temperature and rainfall as environmental factors that can strongly affect phenological parameters.

A significant difference was observed between different habitats in terms of morphological and yield traits. The value of seed length and diameter, fruit dry weight, 1000-seed weight, and fruit length had the highest values in Anbarabad habitat and had a significant difference with other habitats ($p<0.05$). It can be interpreted that increasing the number of days until maturity will reduce the 1000-seed weight. Finally, the reduction of these traits will cause the yield because there is a positive correlation between them and yield. Similar results have been reported for barberry species by other researchers (Goodarzi *et al*., 2018; Tatari *et al*., 2019). Jannatizadeh and Khadivi-Khub (2016) observed a significant phenotypic diversity between the studied species of barberry. A significant correlation was found for the variables related to seed and fruit, so the amount of seed, 1000-seed weight, and seed length positively correlated with the barberry yield.

Trait correlation analysis is used to investigate and establish meaningful relationships between traits. Establishing this relationship between traits leads to studying traits that may be difficult to measure (Rezaei *et al*., 2020; Farrokhi *et al*., 2021). Simple correlation coefficients between traits showed that some measured traits have a significant positive or negative correlation. Barberry yield has a positive and significant correlation with yield components and physiological characteristics such as chlorophyll content. This shows that more plant photosynthesis and primary metabolites will be produced by increasing the amount of leaf chlorophyll. This issue will increase growth parameters, including height and root yield. By increasing the root yield, the production of secondary metabolites will be expanded, the qualitative yield of this plant will be increased, and finally, the product yield will be increased (Kramer, 2012). In this regard, Rezaei *et al*. (2018) investigated the morphological characteristics of seedless barberry. They stated that quantitative and qualitative attributes such as color, size and shape, 1000-seed weight, and titratable acidity vary significantly among the studied regions. Khodabandeh *et al*. (2022) evaluated some morphological characteristics (including panicle length, number of fruits in a panicle, 1000-seed weight, fruit flesh, and color indices in fresh and dry fruit) 16 barberry genotypes native to Iran. They stated that there is a significant difference between all these traits ($p<0.01$).

Ahmed *et al*. (2013) reported that barberry (*Berberis aristata* DC.) has excellent diversity in phenotypic and morphological traits. In addition, habitat had a significant effect on quantitative parameters. They also stated that not only the genotype but also the habitat of its growth is the main factor determining barberry fruits' growth, productivity, and food composition.

In line with the present study's findings, Talebi *et al*. (2020) reported that the effect of genotype on all studied traits of barberry in the northeast region of Iran was significant. These researchers stated that 'Dergez-2' in terms of leaf number, thorn length, and angle, 'Shirvan-2' in petiole length, 'Shirvan-3' in terms of 100-dry seed weight and panicle length, 'Golestan-5' in terms of seed length to diameter ratio had a significant advantage compared to other genotypes. These researchers concluded that different barberry genotypes had a wide range of morphological characteristics. Also, genetic similarities can be divided into similar groups and used in breeding programs. Ahmed *et al*. (2013) stated that the reason for this variation in different barberry species is the genotype and habitat climatic conditions as well as the geographical location of the growth habitat. Talebi *et al*. (2020) stated that the superiority of one genotype over another in terms of morphological characteristics is due to their genetic and environmental differences.

Regarding the yield characteristics of barberry, the researchers stated that the geometric attributes of the panicle in different genotypes had differences, which are caused by genetic traits and the effects of different environmental conditions on it (Talebi *et al*., 2020; Khodabandeh *et al*.., 2022). On the other hand, researchers stated that the difference in moisture content in different barberry genotypes leads to a significant change in the physical and geometric characteristics of the panicle (Talebi *et al*., 2020). Heydari *et al*. (2009) reported that reproductive traits are critical in determining the relationship and diversity of species in the barberry genus, which the slow evolution of traits can cause compared to vegetative characteristics. Panicle length in barberry is one of the most important traits that affect other features, and the present study had a significant correlation with different morphological and yield. In this regard, Talebi *et al*. (2020) reported similar results. These researchers stated that to produce larger fruit, which is very important in barberry genotypes and has high quality, traits such as panicle length and diameter can be increased.

Based on the results, there was a significant difference between the phytochemical and physiological traits in different habitats, indicating high genetic diversity between the investigated ecotypes. Other researchers also pointed out the great variety of biochemical and physiological traits between different barberry ecotypes (Khodabandeh *et al*., 2022). The mean comparison of pH, titratable acidity (malic acid), TSS, antioxidant capacity (DPPH), total phenol, chlorophyll, and carotenoids of barberry in Jiroft, Anbarabad, Raber, Baft, and Bam habitats in shown Table 5. According to the results, there was a significant difference between the pH in Anbarabad habitat, but no significant difference was observed between Jiroft, Baft, Raber, and Bam habitats. The highest amount of fruit titratable acidity was related to Jiroft habitat, and the lowest value was associated with Bam habitat. Farhadi Chitgar *et al*. (2017) obtained the titratable acidity and pH of seedless barberry cultivated industrially in the country as 5.63 g of malic acid and 2.30, respectively, consistent with the results obtained from the present study. Fruit TSS was the highest in Anbarabad habitat and lowest in Jiroft and Baft habitats. The highest amount of anthocyanin in the fruit was from Jiroft and Raber habitats. The lowest amount of anthocyanin was also observed in Bam habitat. Therefore, the results show that the barberry plant is one of the rich sources of anthocyanin, which, besides having health-giving properties, can also be used as a natural dye. Farhadi Chitgar *et al*. (2017) showed that barberry contains many anthocyanins.

The highest antioxidant capacity (DPPH) and phenolic properties of the whole fruit were observed in Jiroft and Raber ecotypes. The amount of total phenol can be used as a practical parameter in plant breeding. Khromykh *et al*. (2018) stated that the fruits of all barberry species could be a source of available antioxidants. Still, the reduction of antioxidant capacity in different barberry species can be due to the difference in their growing place and the influence of different environmental factors such as height, soil, light, etc. Mahdavi *et al*. (2016) concluded that natural phenolic compounds are secondary plant metabolites that form a large and diverse group of phytochemicals, including simple phenols, lignans, phenylpropanoids, flavonoids, coumarins, and other compounds. Nichols *et al*. (2014) reported that phenols perform important physiological functions in the plant and also stated that increasing the accumulation of phenolic compounds in leaves improves the stability of *Trifolium subterraneum* against drought. Makhafola *et al*. (2016) said there is a direct relationship between the antioxidant activity and the phenolic content of the plant, and there was a significant difference between the anthocyanin values in all five habitats.

The highest amount of chlorophyll and carotenoids were Anbarabad habitat, and the lowest value was observed in Jiroft and Raber habitats. Khayyat *et al*. (2018) investigated the fruit maturity indicators and growth patterns during the harvesting season of seedless barberry in different altitude conditions. They stated that the number of fruits, fresh and dry weight, pH, titratable acidity, TSS, anthocyanin, and total phenols were significantly changed with the progress of the growing season in different weather conditions. Researchers concluded that the qualitative and quantitative differences between the ecotypes could be caused by the difference in the ecological characteristics of the growing areas, such as temperature, humidity, and other soil and geographical factors (Farrokhi *et al*., 2021). Yuan *et al*. (2020) stated that among the most important environmental factors that have a major impact on the quantity and quality of the active ingredients of medicinal plants, we could mention the temperature of the environment, the altitude of the place, and the physical and chemical characteristics of the soil. In our study, Jiroft city has a low altitude (720 m.a.s.l), and due to its low altitude, it has a high temperature.

Conclusions

In general, the results of this study indicate a high genetic diversity to plan for the protection of genetic reserves, which, from different phenological, morphological, chemical, and physiological aspects and the production of medicinal metabolites, help us in determining the strategies of exploitation, modification, and domestication helps. In summary: I. The study of phenological traits showed that the beginning of vegetative growth was earlier in Jiroft and Anbarabad habitats. Still, considering that in the autumn, with the cooling of the air and the shortening of the day length, the leaves fall of all habitats simultaneously, the growth period of this habitat got longer. Also, flowering was concluded earlier in these habitats. But the coloring of the fruit and the physiological maturity of the fruit started with the cooling of the air. II. This research showed diversity among the studied ecotypes regarding morphological and yield traits. In total, the yield and growth traits of Anbarabad ecotypes have the highest value, Jiroft habitat has the lowest value in the mentioned traits, and III.

There was a significant difference between the phytochemical and physiological traits in all five habitats. In general, in Jiroft habitat, the anthocyanin and antioxidant activity were higher than in others. On the other hand, Anbarabad habitat showed significant superiority in pH, TSS, total chlorophyll, and carotenoids compared to other habitats. Finally, the results showed that the soil factors (amount of macro-elements such as nitrogen, phosphorus, and potassium) and climate (longitude and latitude) are considered to be the most important influencing factors on barberry yield.

Authors' Contributions

Conceptualization, ITK, FN, SM, and MS; Data curation, ITK; Formal analysis, ITK and FN; Methodology, SM and MS; Software, ITK and FN; Supervision, FN, SM, and MS; Validation, FN; Writing – original draft, ITK, FN, SM, and MS; Writing – review & editing, FN, SM, and MS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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