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The Morphological Traits of Iranian Native Cyclamens

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Abstract

The cyclamen (Myrsinascea) as the flowers growing in some temperate regions, are of high ecological and botanical importance and endangered as well. Therefore, to provide an opportunity to identify and protect its species, Iranian native cyclamen was studied. Plants were collected (in winter and spring) from three northern provinces of Iran, i.e., Guilan, Mazandaran, and Golestan, and their morphological traits (14 quantitative and 10 qualitative traits) were assessed. Based on the analysis of variance, there were significant differences in some recorded traits, e.g., leaf number, flower number, flower color, and flower and leaf shape, which would allow selecting plants with more optimal traits. The cluster analysis divided the plants into three groups. Accordingly, the plants collected from the Shadan region in Golestan province and those collected from the Sinava and Shahsheshmeh regions in Chalus County, Mazandaran province differed from the plants collected from the other regions. The plants were collected from the Abpari and Lavij regions in Nur County, the Pasand region in Behshahr County, the Qarnabad region in Gorgan County, and the Deylaman region in Siahkal County were placed in the same group due to their similar morphology although they had been collected from three different provinces. The first group included the cyclamens of the Qarnabad region in Gorgan County, the Lavij region in Nur County, the Deylaman region in Siahkal County, the Pasand region in Behshahr County, and the Abpari region in Nur County. These plants had lowly toothed leaf margins, long petals, and few seeds. The second group included the cyclamens of the Shadan region in Kordkuy County. These plants had longer and heavier hypocotyls, larger and wider leaves, and smaller flowers. They showed the highest number of seeds, hypocotyl diameter, length, and weight, flower number, leaf length and width, leaf area, petiole and peduncle length, and peduncle diameter. The third group included the cyclamens of the Sinava and Shahsheshmeh regions in Chalus County. They had the highest petal width but the lowest peduncle diameter, leaf length, width, and area, flower and leaf number, and hypocotyl weight, length, and diameter. The cyclamens native to Iran are highly diverse in morphological traits and they need to be further investigated by cytogenetic, phytochemical, and molecular methods.

Keywords: Cyclamen, Cytogenetic, Hypocotyl, Phytochemistry.

INTRODUCTION

Floristry, which is an integration of science and art, has today a special place in agriculture. The cultivation and propagation of flowers and ornamental plants as apartment plants, flowering pot plants, cut flowers, and green space and open-air plants in high diversity are growing in the world (Farjadi Shakib *et al.,* 2012). Iran has an excellent climatic diversity and is a rich country in the diversity of plant species (Norozi *et al.,* 2019).

Cyclamen is a genus of ornamental plants, which has been categorized in the family Myrsinacea in recent years and has been cultivated in some moderate regions (Curuk *et al.,* 2015). This genus has 22 species based on Gray-Wilson's (1988) classification and 21 species based on Yesson and Culham's (2006) classification. The genus of cyclamen is relatively small but with attractive flowers. The important genera of cyclamen have drawn attention in the last 85 years (Curuk *et al.,* 2015). Cyclamens are invaluable plants in the horticulture industry with big demand for which as a pot plant or cover plant in green spaces around the world (Farjadi Shakib *et al.,* 2012). Wild cyclamens are listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), so it is necessary to conduct protective research on this genus, which is a precious genetic source of ornamental plants (Curuk *et al.,* 2015). Wild cyclamens have characteristics like freezing resistance, chilling tolerance, flower aroma, flower color, leaf shape, flowering in the autumn and winter, and resistance to pathogens, which are absent in commercial cyclamens, so its species are used to improve these traits in commercial cyclamens (Alaei *et al.,* 2006).

Cyclamen species are perennial plants with fleshy hypocotyls. The leaves have long petioles developing from hypocotyls. They are elliptical, heart-shaped, or spherical with margins toothed to different degrees (Aalaei *et al.,* 2006). Flowers grow on leafless scapes. They have five petals, which are reflex and can be white, red, light red to dark pink, carmine red, and dark red. There are spots on the petals in some species. The fruits are encapsulated, which fall wrapped in peduncle to the ground. The roots grow on the hypocotyl surface or at the bottom of the hypocotyl (Curuk *et al.,* 2015). Cyclamen species have an annual cycle of growth and dormancy and it is not seen in hot seasons since its shoots are dried (Lazarevic and Lazarevic, 2010). Wild cyclamens are distributed in the Meditteranean region (Lebanon, Syria, Turkey, Cypress, Greece, Israel, and Iran) and North Africa (Dole and Wilkins, 1999).

The cyclamens that grow in Iran have different flowering seasons ranging from early December to early April (Alaei *et al.,* 2006). Since there is not an adequate understanding of wild cyclamen and its species or subspecies in Iran (which may have optimal traits for breeding programs and development of new cultivars), it seems necessary to study the morphological, phytochemical, and cytogenetic traits of wild cyclamens to find out their genetic diversity and the affinity of the species.

MATERIALS AND METHODS

We first marked cyclamen habitats, which were specified in Iran's flora, in 2016-2017 and 2017-2018. They were located in the provinces of Guilan, Mazandaran, and Golestan. Then, the plants were collected along with their hypocotyls in the winter and spring from the Deylaman region in Siahkal County, Guilan province, the Sinava and Shahcheshmeh regions in Chalus County, Mazandaran province, the Abpari and Lavij regions in Nur County, Mazandaran province, the Pasand region in Behshahr County, Mazandaran province, the Shadan region in Kordkuy County, Golestan province, and the Qarnabad region in Gorgan County, Golestan province. The geographical locations of the studied sites were recorded with a handheld GPS (Table 1). About 80 plants were collected from each region and they were measured on the site and at the best flowering time (from late February to mid-March).

Table 1. Geographical position of the sampling stations.

Station	Geographical position	
	N	E
Deylaman	36° 53' 17"	49° 54' 47"
Sinava	36 37' 45"	51° 23' 46"
Shahcheshmeh	36° 41' 42"	51° 18' 00"
Abpari	36° 28' 39"	51° 54' 51"
Lavij	36° 22' 28"	52° 02' 18"
Pasand	36° 41' 01"	53° 36' 50"
Qarnabad	36° 49' 23"	54° 35' 25"
Kordkuy	36° 45' 31"	54° 07' 22"

There are no descriptive lists for all cyclamen species in UPOV and IPGRI. We assessed 14 quantitative traits and 10 qualitative traits. They included flower number, petal length, the highest mid-petal width, peduncle length, petal's colored halo, petal shape, peduncle diameter, petal color, leaf number, leaf length, leaf width, leaf area, leaf color, leaf shape, leaf tip shape, leaf serrulation, hypocotyl length, hypocotyl diameter, seed number per plant, petal length, petiole color, and leaf back color.

Hypocotyl

To study the hypocotyls, three healthy hypocotyls were randomly collected from each region, and their diameter, length, and weight were recorded. The diameter was measured with a caliper in cm, the length (thickness) was measured with a caliper from the base to the tip of the hypocotyl in cm, and the weight was determined with a digital scale in g (the average of the three hypocotyls was used in statistical calculations).

Leaf

Leaf length, widths, number, and area were measured for which three leaves were randomly excised from each plant, and their length and width were measured with a ruler in cm. Then, the same leaves were used to measure leaf area. (The averages of the three leaves were used in statistical calculations).

Leaf surface color

Three plants were selected from each region and three leaves were cut from each one. The plants were categorized into the following five classes in terms of leaf surface color, which is a qualitative trait (Fig. 1):

1. Two-thirds of the leaf is light green and one-third is dark green (Class 1);
2. Half is light green and half is dark green (Class 2);
3. Two-thirds is dark green and one-third is light green (Class 3);
4. The leaf surface is mostly dark green with a few light-green spots distributed across it (Class 4);
5. The leaf surface is mostly light green with a few dark-green spots distributed across it (Class 5).

Leaf back color

The plants were classified into the following four classes in terms of the color of the leaf back, which is a qualitative trait (Fig. 2):

1. Grayish green (Class 1);
2. Brownish green (Class 2);
3. Puce (Class 3);
4. Dark violet (Class 4).

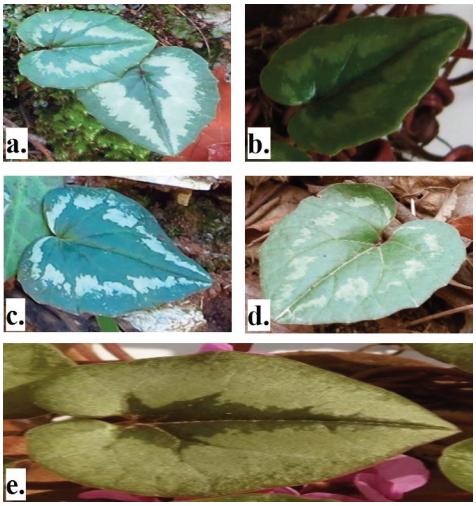

Fig. 1. Five classes of the leaf surface color in the study area: (a.) Class 1, 2/3 of the leaf is light green and 1/3 dark green; (b.) Class 2, half of the leaf is light green and the other half dark green; (c.) Class 3, 2/3 of the leaf is dark green and 1/3 light green; (d.) Class 4, mostly dark green leaf with a few light-green distributed spots; (e.) Class 5, mostly dark green leaf with a few light-green distributed spots.

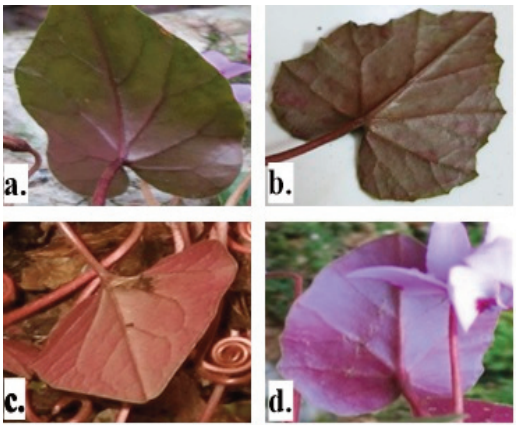

Fig. 2. Four classes of the leaf back color in the study area: (a.) Class 1, Grayish green; (b.) Class 2, Brownish green; (c.) Class 3, Puse ; (d.) Class 4, Dark violet.

Leaf shape

Three plants were selected from each region and their three leaves were randomly cut. The leaves were divided into the following two shape classes (Fig. 3):

1. The leaf ends (lobes) do not overlap and are separate (Class 1);
2. The leaf ends (lobes) overlap (Class 2).

Leaf serrulation

The leaves were classified into the following four classes in terms of their serrulation (Fig. 4):

1. Leaves with smooth margins (Class 1);
2. Leaves with lightly toothed margins (Class 2);
3. Leaves with highly toothed margins and a sharp tip (Class 3);
4. Leaves with heavily toothed margins and in folded shape (Class 4).

Leaf tip shape

Three plants were selected from each region and three leaves were randomly excised from each plant. They were divided into the following three classes in terms of leaf tip (Fig. 5):

1. A common tip, i.e., the tip is neither completely sharp nor completely round (Class 1);
2. The leaf tip is round and semicircular (Class 2);
3. The leaf tip is very sharp and narrow (Class 3).

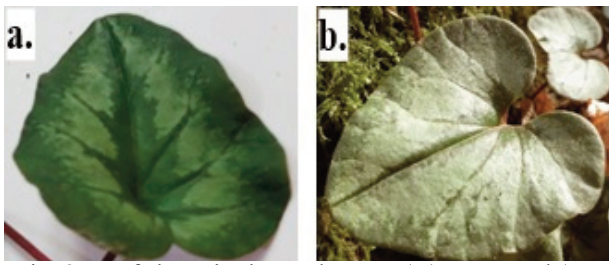

Fig. 3. Leaf shape in the study area: (a.) Separated (no overlapped) ends (lobes) ; (b.) Overlapped ends (lobes).

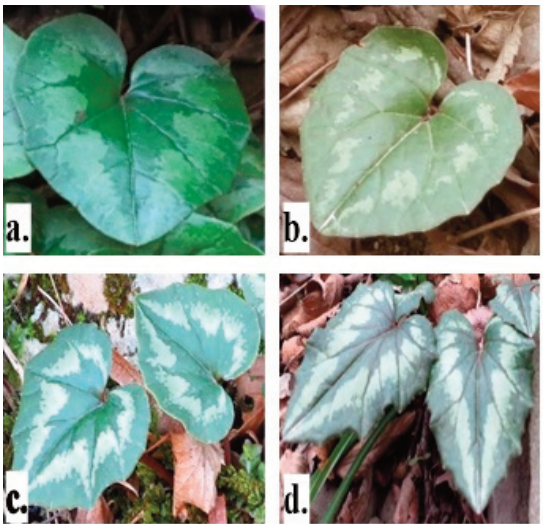

Fig. 4. Four classes of the leaf serrulation in the study area: (a.) Class 1, Smooth margins; (b.) Class 2, Lightly toothed margins; (c.) Class 3, Highly toothed margins and a sharp tip; (d.) Class 4, Heavily toothed margins and in a folded shape.

Petiole length

Petiole length was measured with a ruler in cm on three randomly selected petioles. The petioles were collected from three plants in each region. The recorded lengths of the three petioles were averaged to be used in statistical calculations.

Petiole color

The petioles whose length was measured were divided into the following four classes in color (Fig. 6):

1. Light brown (Class 1);
2. Brownish red (Class 2);
3. Puce (Class 3);
4. Light and pale violet (Class 4).

Fig. 5. Three classes of the leaf tip shape in the study area: (a.) Class 1, Common tip (neither completely sharp nor completely round); (b.) Class 2, Round and semicircular tip (c.) Class 3, Very sharp and narrow tip.

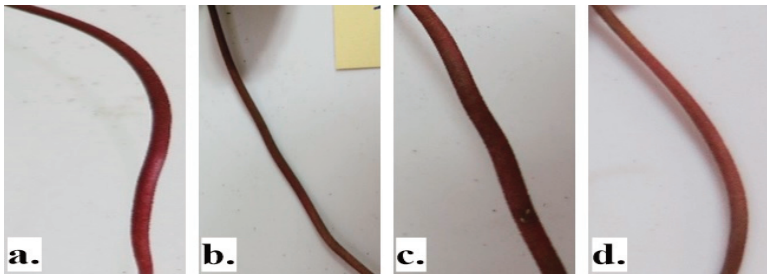

Fig. 6. Four classes of the petiole color in the study area: (a.) Class 1, Light brown; (b.) Class 2, Brownish red; (c.) Class 3, Puce; (d.) Class 4, Light and pale violet.

Flower number

Three plants were randomly selected from each region and their flowers were counted. The averages of each three plants were used in statistical analysis.

Petal size

Three plants were selected from each region, and three flowers were randomly taken from them. Petal length and width (the widest part at the middle of the petal) were measured with a ruler and they were averaged to be used in statistical analysis.

Petal color

Three plants were randomly picked from each region. Then, three flowers were randomly selected from each plant and their petal color, which is a qualitative trait, was placed in one of the following classes (Fig. 7):

1. Light lilac (Class 1);
2. Reddish lilac (Class 2);
3. Pale lilac (Class 3);
3. Dark violet lilac (Class 4).

Petal's colored halo

The colored halo of the petals of the flowers whose color was assessed was divided into the following three classes (Fig. 8):

1. The petals whose darker part (halo) was continuous and triangular (Class 1);
2. The petals whose darker part (halo) was continuous but not triangular (Class 2);
3. The petals whose darker part (halo) was linear with a small area (Class 3).

Petal shape

Three plants were randomly taken from each region and three flowers were randomly selected on each plant. Then, their petal shape was placed in one of the following four classes (Fig. 9):

1. Elongated petal (Class 1);
2. Extended and triangular petal (Class 2);
3. Wavy and extended petal (Class 3);
4. Wavy and elongated petal (Class 4).

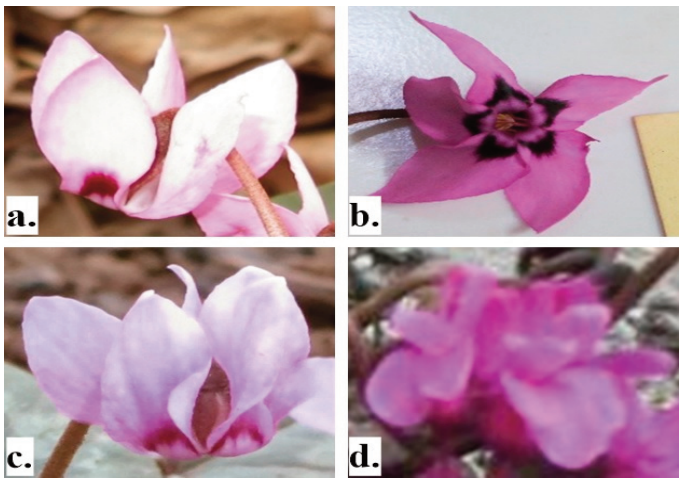

Fig. 7. Four classes of the petal color in the study area: (a.) Class 1, Light lilac; (b.) Class 2, Reddish lilac; (c.) Class 3, Pale lilac; (d.) Class 4, Dark violet lilac.

Fig. 8. Three classes of the petal's colored halo in the study area: The darker part (halo) is (a.) Class 1, Continuous and triangular; (b.) Class 2, Continuous but not triangular; (c.) Class 3, Linear with a small area.

Seed number per plant

Three healthy fruits were randomly taken from each plant to count their seeds. The seeds of the three plants were averaged to be used in statistical analysis.

Statistical analysis

All qualitative and quantitative morphological traits of all samples were measured and recorded in MS-Excel. They were encoded using Windows-based Unistats and analyzed by SAS. The SPSS21 software package was employed to determine the minimums and maximums.

RESULTS

Flower number

Based on the results of the analysis of variance (ANOVA), the collection site had a significant ($P < 0.01$) effect on the flower number of the cyclamens (Table 2). The comparison of the means revealed that the highest number of flowers (39 flowers) was related to the Shadan region, Kordkuy County, and the lowest (4.67 flowers) was observed in the Shahcheshmeh region, Chalus County (Fig. 10.a).

Petal length

The results of ANOVA revealed the significant ($P < 0.01$) effect of the collection site on the petal length of the cyclamens (Table 2). The longest petals were 2.09 cm long observed in the

Fig. 9. Four classes of the petal shape in the study area: (a.) Class 1, Elongated; (b.) Class 2, Extended and triangular; (c.) Class 3, Wavy and extended; (d.) Class 4, Wavy and elongated.

Lavij region, Nur County. The shortest ones were 1.55 cm long collected from the Shadan region, Kordkuy County although this region did not differ from the other regions significantly and all were classified in the same group (Fig. 10b).

The highest mid-petal width

As it was revealed by ANOVA, this trait was not significantly affected by the collection sites (Table 2). According to the comparison of means, there were no significant differences in the highest mid-petal width. However, the highest value was 1.14 cm observed in the Deylaman region, Siahkal County and the lowest was 0.88 observed in the Shadan region, Kordkuy County, and 0.89 cm observed in the Lavij region, Nur County.

Peduncle length

ANOVA showed that the collection site did not influence peduncle length significantly (Table 2). The comparison of the means revealed that the plants collected from the Shadan region, Kordkuy County had the highest peduncle length of 24.722 mm, and those collected from the Lavij region, Nur County, and the Shahcheshmeh region, Chalus County had the lowest one of 12.811 and 13.211 mm, respectively.

Peduncle diameter

The effect of the collection site was found by ANOVA to be significant ($P < 0.01$) on the peduncle diameter (Table 2). The comparison of means showed that the plants collected from the Shadan region, Kordkuy County had the highest peduncle diameter of 2.91 mm, and those collected from the Shahcheshmeh region, Chalus County had the lowest one of 1.028 mm.

Petal color

Based on the results of the Kruskal-Wallis test, the petal color of cyclamens was affected by the collection site significantly ($P < 0.01$). The petal color was divided into four classes as it was described in Materials and Methods.

The comparison of the means showed that the petals in the Deylaman region, Siahkal County were mostly pink and the darkest petals belonged to Class 4 and those in the Sinava and Shahcheshmeh regions, Chalus County were light lilac where the lightest petals belonged to Class 1 (Fig. 11).

Petal's colored halo

The results of the Kruskal-Wallis test showed the significant ($P < 0.01$) effect of the collection site on the petal's colored halo. Based on the comparison of the means, the petal halos in the Shahcheshmeh region, Chalus County were linear belonging to Class 3 and those in the Abpari region in Nur County, the Pasand region in Behshahr County, the Qarnabad region in Gorgan County, and the Shadan region in Kordkuy County were triangular belonging to Class 1 (Fig. 11). Different (three) classes of petal's colored halo are described in the previous section.

Petal shape

The Kruskal-Wallis test showed that the effect of the collection site was significant ($P < 0.01$) on the petal shape of the cyclamens. The petals were wavy and elongated in Class 4 in the Pasan region in Behshahr County and the Lavij region in Nur County.

The petals were elongated belonging to Class 1 in the Abpari, Shadan, Deylaman, and Qarnabad regions (Fig. 11). Petal shape classes are described in the previous section.

Peduncle color

The peduncle color was influenced by the collection site significantly ($P < 0.01$) based on the Kruskal-Wallis test. Based on the comparison of means, the peduncle color was in Class 4 in the Qarnabad region in Gorgan County, the Shadan region in Kordkuy County, the Deylaman region in Siahkal County, and the Pasand region in Behshahr County, so they were light and pale violet, whereas the other regions did not differ significantly (Fig. 11). For the different classes please see the Materials and Methods.

Leaf number

According to the results of ANOVA, the effect of the collection site was significant ($P<0.05$) on the leaf number of the cyclamens. The comparison of the means showed that the highest leaf number (33.47 leave) was related to the Shadan region, Kurdkuy County followed by the Pasand region in Behshahr County where the cyclamens had 30.67 leaves. These two regions were not significantly different. The cyclamens in the Shahcheshmeh region in Chalus County exhibited the fewest number of leaves (7 leaves) (Fig. 13).

Leaf length

Based on the results of ANOVA, the effect of the collection site was not significant on the leaf length of the cyclamens. According to the comparison of the means, the highest leaf length was 5.06 cm measured in the Shadan region, Kordkuy. In Behshahr County, the Pasand region

Table 2. Results of ANOVA of the morphological variables of cyclamen in the study area.

S.o.V	df	Seed number per plant	Hypocotyl diameter	Hypocotyl length	Hypocotyl weight	Flower number	Leaf number	Leaf length	Leaf area	Petal length	The highest mid-petal width	Leaf width	Petiole length	Peduncle diameter
Treat-ment	7	297.1**	20.80*	1.6**	733.14*	345.5**	246.10*	10.00^{ns}	26.70*	00.08**	00.03^{ns}	20.02**	25.73*	00.58**
Error	16	30.00	00.09	00.17	207.88	35.17	89.68	00.40	80.37	00.01	00.03	00.004	80.17	00.13
CV (%)	-	8.39	18.01	12.94	36.46	34.38	44.79	14.99	30.33	6.73	15.85	15.99	22.08	23.10

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

Table 3. Results of Kruskal-Wallis test of the morphological variables of cyclamen in the study area.

Kruskal-Wallis Test	df	Leaf shape	Leaf back color	Petiole color	Leaf color	Leaf shape	Leaf serrulation	Petals colorered halo	Petal shape	Peduncle color
Chi-Square	7	20.25*	20.25**	21.06**	21.06**	12.96**	21.06**	19.71**	18.9**	19.71**

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

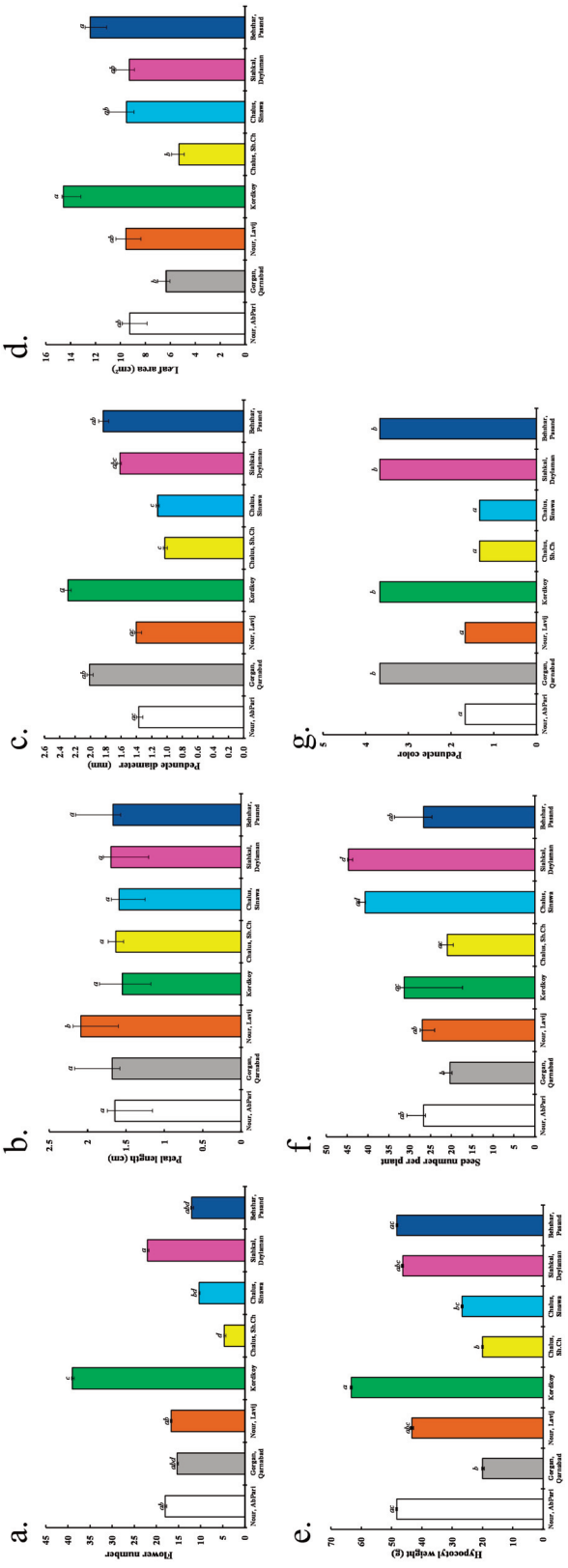

Fig. 10. Flower number (a), petal length (b), peduncle diameter (c), leaf area (d), hypoctyl weight (e), seed number per plant (f), and peduncle color (g) in eight stations of the study area.

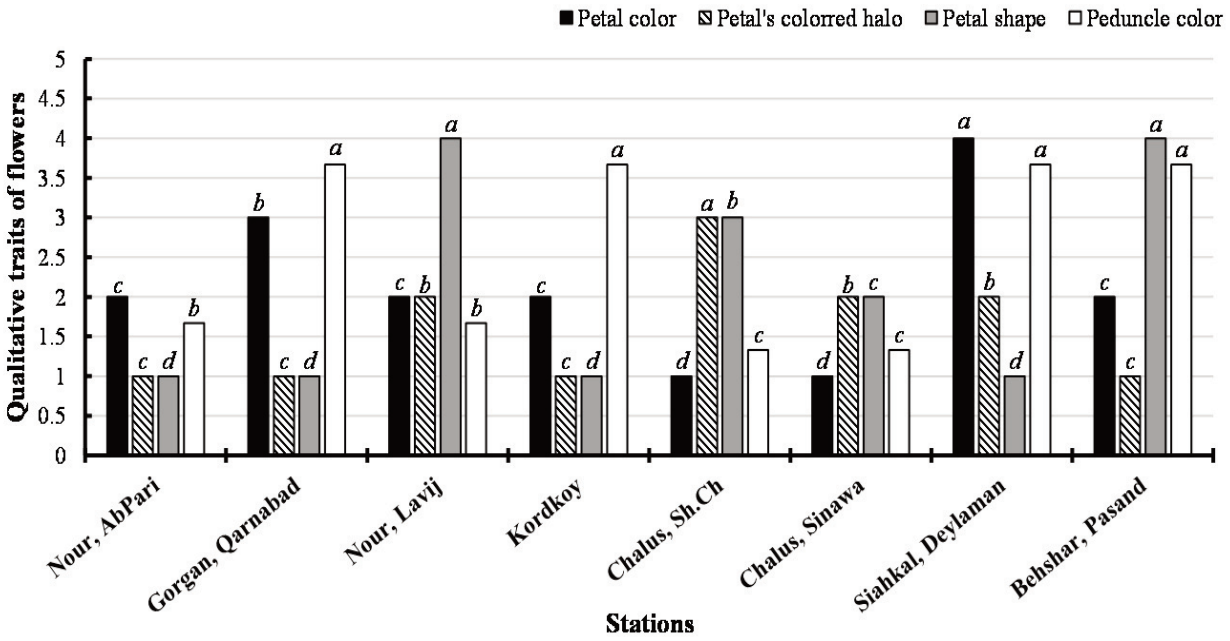

Fig. 11. Petal color, petal's colored halo, petal shape, and petal peduncle of the flowers in eight stations of the study area.

also had longer leaves (4.72 cm) than the other regions and was in the same statistical group as the Shadan region. The lowest petal length was 3.39 cm related to the Shahcheshmeh region in Chalus County (Fig. 13).

Leaf width

The results of ANOVA revealed the significant effect of the collection site on the leaf width of the cyclamens at the $P < 0.01$ level. The highest leaf width (5.70 cm) was related to the Shadan region in Kordkuy County and the lowest (3.02 cm) was measured in the Shahcheshmeh region in Chalus County as the comparison of the means showed (Fig. 13).

Leaf area

The effect of the collection site was found to be significant ($P < 0.05$) on the leaf area of the cyclamens as the results of ANOVA showed. Based on the comparison of the means, the plants collected from the Shadan region in Kordkuy and those collected from the Pasand region in Behshahr had the highest leaf areas of 14.59 and 12.43 cm, respectively. The lowest leaf area was 5.30 cm observed in the Shahcheshmeh region in Chalus County, and it did not differ from that in the Qarnabad region in Gorgan County (6.33 cm) significantly (Fig. 13).

Leaf color

Based on the results of the Kruskal-Wallis test, the leaf color of cyclamens was influenced by the collection site significantly ($P < 0.01$). The comparison of the means revealed that the leaf color did not differ significantly between the Sivana region in Chalus and the Qarnabad region in Gorgan. Recorded in Class 4, the leaves in these two sites were mostly dark green with light green spots distributed across them. The leaves collected from the Abpari region in Nur, the Shadan region in Kordkuy, and the Pasand region in Behshahr did not differ significantly in leaf color where two-thirds of the leaves were light green and one-third was dark green (Class 1) (Fig. 13). In the previous section leaf color classes were described.

Petiole length

According to ANOVA, the effect of the collection site was significant ($P < 0.05$) on the petiole length of the cyclamens. It was found by the comparison of the means that the highest petiole length (19.17 cm) was related to the Shadan region in Kordkuy County and the lowest (9.16) was related to the Qarnabad region in Gorgan County. However, the Qarnabad region was classified in the same statistical group as the other regions and they did not have significant differences from one another (Fig. 13).

Petiole color

Based on the results of Kruskal-Wallis test, the petiole color of the cyclamens in the study area revealed a significant difference ($P < 0.01$) between the sampling sites (the effect of site was significant, Fig. 13).

Leaf shape

The Kruskal-Wallis test revealed the significant effect of the collection site on the leaf shape of the cyclamens at the $P < 0.01$ level. Based on the comparison of means, the leaf shape did not differ between the Abpari region in Nur County and the Pasand region in Behshahr County significantly where the leaf lobes overlapped (Class 2). Other regions did not show significant differences and all leaves were in Class 1 as the leaf lobes were separate and heart-shaped. Two classes of the leaf shape were introduced in the previous section (Fig. 13).

Leaf tip shape

It was found by the Kruskal-Wallis test that the leaf tip shape of the cyclamens was significantly ($P < 0.01$) influenced by the collection site. The comparison of means showed that the cyclamens in the Sivana region and those in the Shahcheshmeh region did not have significantly different leaf tip shapes so that both belong to Class 3 as their leaves had very sharp tips. Similarly, no significant differences were observed among the cyclamens in the Deylaman region in Siahkal County, the Lavij region in Nur County, and the Abpari region in Nur County and all belonged to Class 1 as their leaves had a common tip. Round and semicircular leaf tips were observed in the cyclamens of the Shadan region in Kordkuy County, the Qarnabad region in Gorgan County, and the Pasand region in Behshahr County (Fig. 13). Three leaf tip classes were introduced in the previous section.

Leaf serrulation

Based on the Kruskal-Wallis test, the effect of the collection site was significant ($P < 0.01$) on leaf serrulation. The comparison of the means revealed that the leaves in the Sinava and Shahcheshmeh regions in Chalus County had no significant difference in this trait so that they were heavily toothed with a folded shape (Class 4). The cyclamens in the Qarnabad region in Gorgan County, the Shadan region in Kordkuy County, and the Pasand region in Behshahr County belonged to Class 1 and their leaves had smooth margins with no teeth (Fig. 13).

Leaf back color

The leaf back color was significantly ($P < 0.01$) affected by the collection site as the Kruskal-Wallis test showed. Based on the comparison of the means, the leaf back color belonged to Class 4 (dark violet) in the Deylaman region in Siahkal, the Shadan region in Kordkuy, and the Abpari region in Nur, differing insignificantly with one another. The leaf back color belonged to Class 2 in the Lavij region in Nur and the Sinava region in Chalus (Fig. 13).

Peduncle color

The Kruskal-Wallis test showed that the collection site influenced this trait significantly ($P< 0.01$). Peduncles in the Deylaman region in Siahkal County were light and pale copper (Class 4) and light and pale purple (Class 1) in the Sinava region and the Shahsheshmeh region in Chalus County. Four classes of the peduncle color are described in Materials and Methods.

Hypocotyl weight

The hypocotyl weight of the cyclamens was found to be influenced by the collection site significantly ($P < 0.05$) as ANOVA showed. Based on the comparison of the means, the cyclamens in the Shadan region in Kordkuy had the heaviest hypocotyls (63.33 g) and those in the Shahsheshmeh region in Chalus and the Qarnabad region in Gorgan had the lightest ones (20.33 g) (Fig. 13).

Hypocotyl length

According to ANOVA, the effect of the collection site was significant ($P < 0.01$) on the hypocotyl length of the cyclamens. The comparison of the means showed that the cyclamens in the Shadan region, Kordkuy had the longest hypocotyls (4.00 cm), and those in the Shahsheshmeh region, Chalus had the shortest ones (2.20 cm) (Fig. 13).

Hypocotyl diameter

The results of ANOVA revealed that the effect of the collection site was significant ($P<0.05$) on the hypocotyl diameter of the cyclamens. It was found by the comparison of the means that the hypocotyl diameter was the highest (6.50 cm) in the Shadan region, Kordkuy County, and the lowest in the Shahsheshmeh region (4.07 cm) and the Sinava region (4.47 cm), Chalus County. The Shahsheshmeh region and the Sinava region did not differ in this trait significantly (Fig. 13).

Seed number per plant

ANOVA showed that the effect of the collection site was significant ($P < 0.01$) on the seed number of the cyclamens. Based on the comparison of the means, the highest number of seeds per plant was, on average, 44.67 related to the cyclamens in the Deylaman region in Siahkal and the lowest number was, on average, 20.33 for the cyclamens in the Qarnabad region in Gorgan.

Cluster analysis of morphological traits

Based on all recorded morphological traits, the plants in different sites were divided into three groups. This grouping shows that the plants in the Shadan region in Kordkuy, Golestan, and the plants in the Sinava and Shahsheshmeh regions in Chalus, Mazandaran are different from those in other sites. The plants of the Abpari and Lavij regions in Nur, the Pasand region in Behshahr, the Qarnabad region in Gorgan, and the Deylaman region in Siahkal were placed in the same group due to their morphological similarities although they were in different provinces.

Group 1 includes the cyclamens in the Qarnabad region in Gorgan, the Lavij region in Nur, the Deylaman region in Siahkal, the Pasand region in Behshahr, and the Abpari region in Nur. These plants are similar in leaf serrulation so that they are all lowly toothed. Also, they are similar in having long petals and a low number of seeds.

Group 2 is composed of the cyclamens in the Shadan region in Kordkuy. These plants have long and heavy hypocotyls, larger and wider leaves, and smaller flowers. Although these plants were not unique in terms of the studied qualitative traits, their vegetative parts were larger than those of the plants in other regions and this might be the reason for their classification as a separate group. The cyclamens in the Kordkuy region had the highest number of seeds, hypocotyl diameter, length, and weight, flower number, leaf number, length, width, and area, petiole length, and peduncle length and diameter.

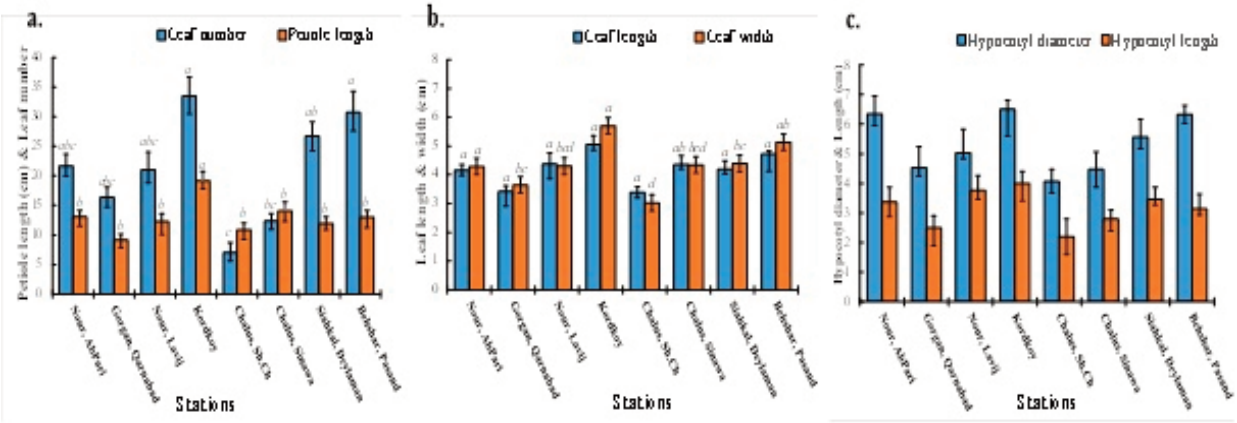

Fig. 12. Petiole length, number of leaves (a), length and width of leaves (b), and hypocotyl diameter and length (c) in eight stations of the study area.

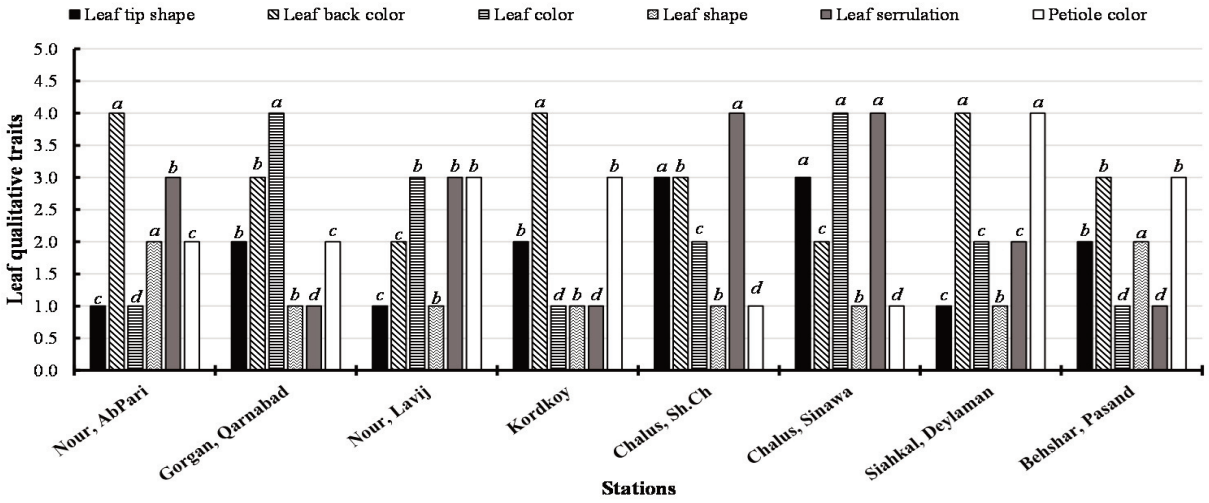

Fig. 13. Leaf quality traits: Leaf tip status, leaf back color, leaf color, leaf serrulation, leaf shape, and petiole color in eight stations of the study area (for different classes of each variable, please see materials and methods).

Group 3 includes the Sinava and Shahsheshmeh regions in Chalus. The plants in this group had the widest petals but the lowest peduncle diameter, leaf length, width, and area, flower and leaf number, and hypocotyl weight, length, and diameter. So, unlike the plants of Kordkuy, they should have a smaller appearance than the other studied cyclamens. In terms of qualitative traits, their petals are light lilac and their leaves are piebald and dark with a sharp tip, highly toothed, and folded in the margins. The lobes of the leaves are completely separate and the petioles are light brown (Fig. 14).

Correlation of morphological traits

Correlation of quantitative morphological traits

According to the coefficients of correlation (Table 4), the quantitative morphological trait of seed number was positively and significantly ($P < 0.05$) correlated to the hypocotyl length. Also, the hypocotyl diameter was positively correlated to the hypocotyl length and weight and leaf number at the $P < 0.01$ level and to the flower number and leaf length at the $P < 0.05$ level. The results showed

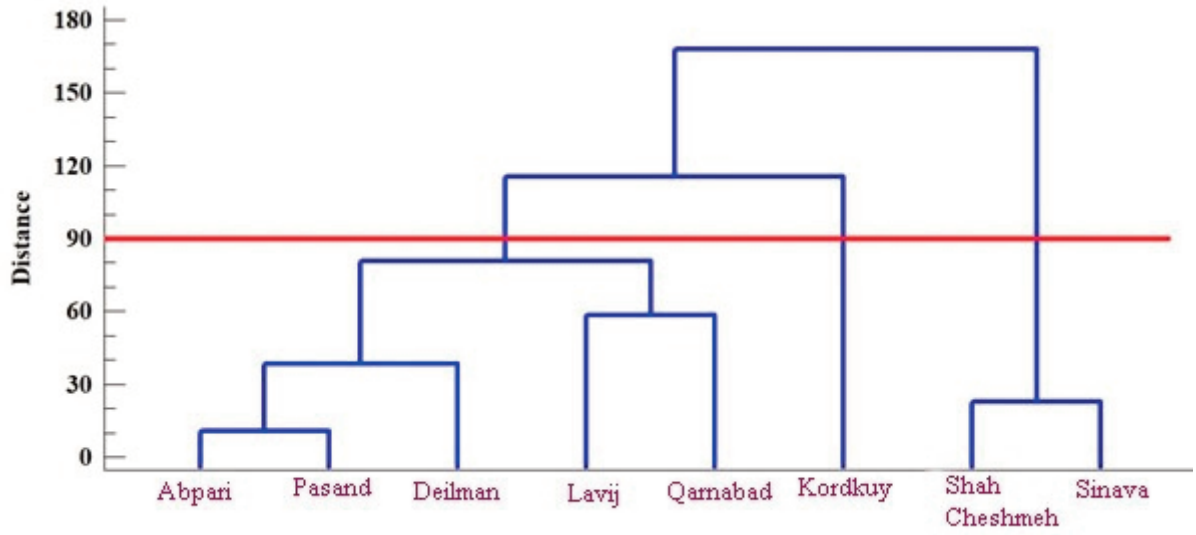

Fig. 14. Grouping of studied cyclamens using cluster analysis based on measured morphological traits.

a significant ($P < 0.01$) correlation of the hypocotyl length with the hypocotyl weight and leaf number, length, width, and area. The hypocotyl weight showed a significant ($P < 0.01$) positive correlation with the flower and leaf number, leaf length, width, and area, and peduncle length. The flower number was positively correlated with the leaf number and peduncle ($P < 0.01$) whereas its correlation was positive with the leaf width and peduncle length and negative with the widest leaf width ($P < 0.05$). Based on the coefficient of correlation, the correlation of the leaf number was significant with the peduncle diameter at the $P < 0.01$ and with the leaf width at the $P < 0.05$ level. The leaf length showed a significant positive correlation with the leaf area and width and peduncle length at the $P < 0.01$ level and with the peduncle diameter at the $P < 0.05$ level. The leaf area was found to have a significant ($P < 0.01$) positive correlation with the leaf width, peduncle length, and peduncle diameter. The correlation of the leaf width was positive and significant ($P < 0.01$) with the peduncle length and diameter. The petiole length and peduncle length were correlated positively at the $P < 0.01$ level. Based on the results, a positive correlation ($P < 0.01$) was detected between the peduncle length and diameter. But, the petal length had no significant correlation with any studied traits.

Correlation of qualitative morphological traits

The coefficients of correlation (Table 5) showed that the leaf tip shape had a negative correlation ($P < 0.01$) with the petiole and petal color. The leaf back color distribution was correlated with the leaf color and petal shape negatively ($P < 0.01$) and with the petiole, petal, and peduncle color positively ($P < 0.05$) whereas its correlation was negative ($P < 0.05$) with the leaf serrulation and petal's colored halo. The correlation of petiole color was positive ($P < 0.01$) with the petal and peduncle color and negative ($P < 0.01$) with the leaf serrulation. The leaf color exhibited a negative correlation with the leaf shape at the $P < 0.01$ level and a positive correlation with the petal's colored halo at the $P < 0.05$ level. Also, a negative correlation was detected between the leaf shape and petal's colored halo at the $P < 0.01$ level. The leaf serrulation had a positive correlation with the petal's colored halo and a negative correlation with the petal and peduncle color at the $P < 0.01$ level. A positive correlation was found between the petal color and its shape and a negative correlation was seen between the petal color and peduncle color at the $P < 0.01$ level while the correlation of the petal's colored halo and petal color was negative at the $P < 0.05$ level. The petal's colored halo was negatively correlated with the peduncle color at the $P < 0.01$ level.

Table 4. Results of correlation coefficients of quantitative morphological traits.

	Flower number	Petal length	Peduncle length	The highest mid-petal width	Leaf number	Leaf length	Leaf width	Leaf area	Hypocotyl weight	Hypocotyl length	Hypocotyl diameter	Seed number per plant	Petiole length	Peduncle diameter
Flower number	1.00													
Petal length	-0.07^{ns}	1.00												
Peduncle length	0.53**	-0.32^{ns}	1.00											
The highest mid-petal width	-0.41*	0.20^{ns}	0.20^{ns}	1.00										
Leaf number	0.70**	0.09^{ns}	0.39^{ns}	-0.08^{ns}	1.00									
Leaf length	0.34^{ns}	-0.08^{ns}	0.66**	-0.03^{ns}	0.31^{ns}	1.00								
Leaf width	0.46*	-0.08^{ns}	0.71**	-0.04^{ns}	0.41*	0.88**	1.00							
Leaf area	0.40^{ns}	-0.12^{ns}	0.67**	-0.02^{ns}	0.34^{ns}	0.94**	0.97**	1.00						
Hypocotyl weight	0.68**	-0.04^{ns}	0.49**	-0.19^{ns}	0.64**	0.57**	0.60**	0.59**	1.00					
Hypocotyl length	0.74**	0.18^{ns}	0.37^{ns}	-0.30^{ns}	0.71**	0.52**	0.53**	0.52**	0.80**	1.00				
Hypocotyl diameter	0.49*	-0.06^{ns}	0.34^{ns}	-0.28^{ns}	0.55**	0.31^{ns}	0.44*	0.36^{ns}	0.77**	0.56**	1.00			
Seed number per plant	0.34^{ns}	-0.18^{ns}	0.19^{ns}	0.08^{ns}	0.35^{ns}	0.27^{ns}	0.15^{ns}	0.15^{ns}	0.26^{ns}	0.40*	0.03^{ns}	1.00		
Petiole length	0.48*	-0.04^{ns}	0.68**	0.04^{ns}	0.33ns	0.65**	0.68**	0.69**	0.51**	0.30^{ns}	0.30ns	0.14^{ns}	1.00	
Peduncle diameter	0.54**	-0.15^{ns}	0.27^{ns}	0.05^{ns}	0.53**	0.43*	0.57**	0.56**	0.33^{ns}	0.39^{ns}	0.16ns	-0.01^{ns}	0.56**	1.00

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

Table 5. Results of correlation coefficients of morphological qualitative traits.

Traits	Leaf tip shape	Leaf back color	Petiole color	Leaf color	Leaf shape	Leaf serrulation	Petal color	Petal's colored halo	Petal shape	Peduncle color
Leaf tip shape	1.00									
Leaf back color	-0.39^{ns}	1.00								
Petiole color	-0.71**	0.41*	1.00							
Leaf color	0.27^{ns}	-0.69**	-0.38^{ns}	1.00						
Leaf shape	-0.27^{ns}	0.27^{ns}	0.07^{ns}	-0.65**	1.00					
Leaf serrulation	0.27^{ns}	-0.42*	-0.62**	0.33^{ns}	-0.20^{ns}	1.00				
Petal color	-0.69**	0.49*	0.74**	-0.04^{ns}	0.00^{ns}	0.69**	1.00			
Petal's colored halo	0.26^{ns}	-0.41*	-0.26^{ns}	0.43*	-0.55**	0.78**	-0.41*	1.00		
Petal shape	0.22^{ns}	-0.72**	-0.08^{ns}	0.07^{ns}	0.14^{ns}	0.28^{ns}	0.49**	0.38^{ns}	1.00	
Peduncle color	-0.20^{ns}	0.44*	0.62**	-0.24^{ns}	0.04^{ns}	0.85**	0.68**	-0.53**	-0.32^{ns}	1.00

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

DISCUSSION

Cyclamen species are endangered, despite their high potential for ornamental applications, due to the destruction of their natural habitats, the uninformed use of agricultural lands, and the translocation of their hypocotyls for exporting, so it is necessary to conduct conservative studies on cyclamens, which are an invaluable genetic source of ornamental plants (Curuck *et al.,* 2015).

On the other hand, plant breeding researchers emphasize the significance of using germplasm of wild landraces due to their characteristics such as resistance to adverse environmental conditions, resistance to pests and diseases, and their high active ingredient contents (Saeedi *et al*., 2013).

This study reports the morphological traits of cyclamens growing wildly in Iran. Some morphological traits, e.g., high chilling resistance, having attractive leaves, or having a high number of flowers, are interesting and can be used in breeding studies or research on developing new interspecific hybrids in the future (Debussche and Thomeson, 2002). We found that the cyclamens that had grown in the Kordkuy region had higher potential than the others. For example, the plants grown in some habitats had similar traits, e.g., high flower and leaf numbers and high leaf area, to the wild species studied in other countries.

In terms of the leaf color, the cyclamens grown in the Qarnabad region, Gorgan and the Shahsheshmeh region, Chalus were similar to the wild species *C. graecum* and *C. alpinum* and those collected from the Abpari region in Nur, the Shadan region in Kordkuy, and the Pasand region in Behshahr were similar to the wild species *C. hederifolium* (Curuck *et al.,* 2015).

The cyclamens collected from the Abpari region and the Pasand region had a similar leaf shape to the wild species *C. graecum* and those collected from the Qarnabad region in Gorgan, the Lavij region in Nur, the Shadan region in Kordkuy, the Sinava and Shahsheshmeh regions in Chalus, and the Deylaman region in Siahkal had similar leaf shape to the wild species *C. hederifolium*, *C. mirabile*, and *C. alpinum* (Curuck *et al*., 2015). In terms of the leaf tip shape, the plants in the Sinava and Shahsheshmeh regions in Chalus had similarities were *C. hederifolium* and *C. graceum*, those in the Deylaman region in Siahkal and the Abparia and Lavij regions in Nur were similar to *C. mirabile*, and those collected from the Shadan region in Kordkuy, the Qarnabad region in Gogran, and the Pasand region in Behshahr were similar to *C. alpinum*. The cyclamens in the Deylaman region, Siahlak had similar petal color to *C. alpinum* and those grown in the Sinava and Shahsheshmeh regions in Chalus were similar to *C. mirabil* (Curuck *et al*., 2015). The petal shape of the cyclamens collected from the Pasand region in Behshahr and the Lavij region in Nur was similar to *C. hedrerifolium* (Curuck *et al*., 2015).

The similarities of Iranian native cyclamens with some other species of cyclamen in some morphological traits may imply that there are many species or subspecies of cyclamens in Iran, which need further cytogenetic and molecular investigation. The morphological investigation of cyclamens in Iran and the classification of their traits will help such efforts as germplasm protection programs in protecting endangered cyclamens.

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